Gregarines of the barnacles from Puget Sound and adjacent areas [1].

By

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With plate 22.

Introduction.

Many species of barnacles have been examined by different workers for their gregarine parasites and several new species have been described. Unfortunately because of the meager descriptions, which are not always accompanied by figures of the gregarines described, the exact systematic position of several of the gregarines can not be ascertained.

KÖLLICKER (1848) was the first to name and figure a gregarine from barnacles. He gave a very brief description of a cephalont of *Gregarina balani* from the intestine of *Balanus pusillus.*

NUSSBAUM (1890) incompletely described *Gregarina valettei* from *Pollicipes polymerus.* He figures both cephalonts and a syzygy.

The status of another gregarine, described by MINGAZZINI (1891) is likewise uncertain. He does not figure but gives a brief description of *Nematoides fusiformis* from the intestine of *Balanus perforatus.* This form is a monocystid gregarine, pointed at both ends, with an epimerite in the form of a cupping glass (ventósa). LABBÉ (1899) emends the description as follows: "Epim. en forme de fourche ou de pince, séparé par un col allongé du reste du corps." LABBÉ states that the cephalont is 38 μ of which 4—5 μ is the epimerite and gives *Pollicipes cornucopia* as a second host. He refers to MINGAZZINI (1893) which is not available, so it is not known whether LABBÉ'S contribu-

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tions are original or whether MINGAZZINI later found this gregarine in another host and gave a different description of the epimerite. Until further work is done, it will have to remain an uncertain species.

The first comprehensive work on the gregarines of barnacles was done by MAWRODIADI in 1908. He described most of the life history of the gregarine first seen by SOLGER (1890) but not named by him. This gregarine for which MAWRODIADI described the new genus *Cephaloidophora*, was given the specific name *communis*. *Ceph. communis* was found in the intestine of several species of *Balanus*. MAWRODIADI placed the *Cephaloidophora* in the family Stenophoridae, principally because of the intracellular development and the lack of an epimerite.

MAWRODIADI also describes but does not name another gregarine occurring with *Ceph. communis* in *Bal. amphitrite*. The sporonts are 130—140 μ in length and the cephalont has a long epimerite which penetrates the entire epithelial layer.

LÉGER and DUBOSCQ (1909) named a gregarine, found by them in *Chthamalus stellatus*, *Frenzelina chthamali*. In 1911 the authors recognized *Frenzelina* as a synonym of *Cephaloidophora*. However, this species was later described by TRÉGOUBOFF (1912) who placed it in a new genus as intracellular development does not occur.

BUDINGTON (1910) gave a brief description of a gregarine, which he did not name or figure, from the intestine of *Bal. eburneus*. From the meager description, it seems possible that he may have seen *Ceph. communis*.

The second real contribution to the study of gregarines in barnacles was that of TRÉGOUBOFF in 1912. He gave a detailed account of the morphology of three gregarines. His description of *Ceph. communis* in general agrees with that of MAWRODIADI but adds a few details.

TRÉGOUBOFF also described a new genus *Pyxinoides*, with two species. *Pyxinoides balani* was found rarely in *Bal. amphitrite* and *Bal. eburneus*. The epimerite has a swollen, globular head, with sixteen projecting flanges and is surmounted by a conical mucron. The head is connected to the protomerite by a neck of varying length, which may remain after the epimerite is lost. Association is precocious and consists of two or at most three associates. There is a great difference in size; the primite is 120—130 μ; the satellite, 60 μ, in length.

TRÉGOUBOFF also transferred *Frenzelina chthamali* LÉGER and DUBOSCQ (1909) to the genus *Pyxinoides* and gives a complete de-

scription. He traced the development of the epimerite from the earliest stages to the final stage in which the epimerite has the form of an upturned cup, the edge of which is cut in eight teeth. It is attached by a short neck which remains fastened to the protomerite after the epimerite is broken off. The protomerite has a clear zone in the form of a cap with a short canal leading to the base of the neck. Association occurs early; the primites are 120—130 μ and the satellites 55—70 μ in length. Occasionally very old associations were found in which the primite was 230 μ and the satellite, 170 μ in length.

TRÉGOUBOFF did not observe the rupture of the cyst or spores in either of these two species.

KAMM (1922) created a new family Cephaloidophoridae with the following diagnosis:

"Intestinal parasites of Crustacea, development intracellular, early syzygies of two sporonts. Cysts without sporoducts, spores ovoidal with equatorial line. Entire life cycle passed within a single host."

In a discussion of the new family, KAMM states that the only important difference between the Cephaloidophoridae and the Stenophoridae, in which family SOKOLOW (1911) placed the *Cephaloidophora*, although MAWRODIADI (1908) had already done so, is that the Cephaloidophoridae invariably form early syzygies, whereas the Stenophoridae invariably do not. As will be shown later, this is not a characteristic of all members of the genus *Cephaloidophora*.

However, as KAMM pointed out, the gregarines of Crustacea should be considered separately because of the different types of Sporozoa which may occur as parasites. For this reason, it seems necessary to retain the family Cephaloidophoridae until more is known about the life cycles of the various gregarines.

KAMM also gives brief descriptions, and figures when available, of the following gregarines of barnacles: *Ceph. communis*, *Pyx. balani*, *Pyx. chthamali*, *Greg. valettei*, and *Nematoides fusiformis*. She believes the *Gregarina balani* of KÖLLICKER is the same gregarine as *Pyx. balani*.

The most recent work on the gregarines of barnacles is that of BALL (1937) who in studying *Ceph. communis* in *Bal. amphitrite* at Séte, France, found cysts and ovoidal sporocysts in chains attached to the appendages and hairs of the body, particularly in the molt. He also formed a new genus *Carcinoecetes* which differs from *Cephaloidophora* in that the intracellular development is brief and the

round to ovoidal sporocysts are not discharged in chains. Both species of this genus were found in *Pachygrapsus*, however.

In undertaking further studies on the gregarines of barnacles, it was hoped that in addition to describing the species occurring in the barnacles of Puget Sound, the complete life cycles of these forms could be ascertained. In addition to studying the living gregarines, the intestines of infected barnacles were fixed and stained slides prepared. Very good results were obtained in determining the early development of the gregarine. Also every effort was made to find the cysts and sporocysts of each species studied. Although cysts have been found in nearly all species, in no case did they complete their development with the formation of sporocysts. It should also be mentioned that, considering the number of mature sporonts that were found, the cysts seen in the faeces or gut were much fewer in number than would be expected. Cysts were not found outside of the gut, except in the faeces.

In the following pages, descriptions of the gregarines found in barnacles of the Puget Sound Region are given with the hope that further work may elucidate the life histories of these forms and that other workers will be stimulated to study these very interesting parasites.

The barnacles which have been examined for gregarines were for the most part from Puget Sound, the waters of the San Juan Archipelago, and the Strait of Juan de Fuca. Approximately twelve hundred barnacles have been examined from a number of different localities. The barnacles in which gregarines were found are *Bal. crenatus*, *Bal. glandula*, *Bal. cariosus*, *Bal. nubilis*, *Bal. balanus pugetensis*, *Bal. rostratus heteropus*, and *Mitella polymerus*.

Cephaloidophora communis Mawrodiadi.

(Pl. 22 Fig. 1.)

This gregarine described by Mawrodiadi in 1908 was again studied by Trégouboff in 1912, who agreed for the most part with the findings of Mawrodiadi but added further details.

Ceph. communis was found in the gut of *Bal. improvisus*, *Bal. eburneus*, and *Bal. amphitrite* by Mawrodiadi. Trégouboff found it in *Bal. tintinnabulum* and *Bal. perforatus* as well as in the above three species.

Ceph. communis has been found quite regularly in the common inter-tidal barnacles of Puget Sound, *Bal. crenatus*, *Bal. glandula*,

and *Bal. cariosus*. The development of the cephalont and growth of the sporont was found to be the same as that described by MAWRODIADI and TRÉGOUBOFF.

In the material examined by the author, cephalonts were released from the epithelial cells when they were 20—24 μ as stated by MAWRODIADI, whereas TRÉGOUBOFF found they were liberated when 35—40 μ in length.

MAWRODIADI found that the sporont of *Ceph. communis* never exceeds 70 μ; he does not figure a sporont. TRÉGOUBOFF states that the difference in length of associates is very pronounced and that the mature primite is rarely over 80 μ and the satellite is 60—65 μ. He also does not figure a sporont.

Ceph. communis in the shore barnacles of Puget Sound associates early; sporonts in association usually range in length from 37—82 μ. Occasionally a few much larger sporonts, both solitary and in association, have been seen. These will be discussed later. There is not a pronounced difference in length between the primite and satellite, although the primite is usually longer but about 40 % of those measured were the same length. The protomerite of the satellite fits into the deutomerite of the primite and as has been stated by BALL (1937), the association is not very securely fastened. The ratio of the length of the protomerite to the total length is as 1 is to 3.5 and the width of the protomerite to the width of the deutomerite is as 1 is to 1.2. The ratio of the greatest width to the total length is as 1 is to 2.4. There is no change in relative size of the protomerite and deutomerite during growth.

A few typical measurements in microns are given below.

		Primite		Satellite	
Length protomerite	10	10	24	10	20
„ deutomerite	24	31	58	31	54
Width protomerite	14	14	27	14	24
„ deutomerite	14	17	37	15	27
Total length sporont	34	41	82	41	74
„ „ association	—	82	156	—	—
Ratio:					
Length proto: total length	3.4	4.1	3.4	4.1	3.7
Width proto: width deut.	1.0	1.2	1.4	1.1	1.1
Greatest width: total length	2.4	2.4	2.2	2.7	2.7

As mentioned above, occasionally sporonts belonging to the genus *Cephaloidophora* and resembling *Ceph. communis* except that they are

much larger, have been found in both *Bal. cariosus* and *Bal. crenatus* (Pl. 22 Fig. 4). There is some possibility that these may represent two new species of the genus *Cephaloidophora*, but the available data is insufficient to form new species at this time. However, a brief description will be given of the two forms to facilitate further work.

The large sporonts, both solitary and in association, have been found in *Bal. cariosus* from four localities in Puget Sound. They resemble *Ceph. communis* in the type of disc at the anterior end of the protomerite and in having a nucleus with numerous karyosomes. The proportions of the different parts are different, however. The ratio of the length of the protomerite to the total length is as 1 is to 3.3, the ratio of the width of the protomerite to the width of the deutomerite is as 1 is to 1.2, and the ratio of the greatest width is to the total length as 1 is to 2.1. The satellite is longer than the primite. A few typical measurements in microns are given below.

			Primite	Satellite
Length protomerite	62	48	55	48
„ deutomerite	117	138	173	197
Width protomerite	90	76	97	97
„ deutomerite	90	83	145	110
Total length sporont	179	186	228	245
„ „ association			473	—
Ratio:				
Length proto: total length	2.9	3.9	4.1	5.0
Width proto: width deut.	1.0	1.1	1.5	1.1
Greatest width: total length	2.0	2.2	1.7	2.2

Large sporonts were also found in *Bal. crenatus* from two different localities, collected in both instances at minus tides. No associatons were seen. The ratio of the length of the protomerite to the total length is as 1 is to 5.0, the ratio of the width of the protomerite to the width of the deutomerite as 1 is to 1.2, and the ratio of the greatest width to the total length as 1 is to 3.2. A few typical measurements in microns are given below.

Length protomerite	22	29
„ deutomerite	97	97
Width protomerite	32	32
„ deutomerite	36	32
Total length sporont	119	126

Ratio:		
Length proto: total length	5.5	4.0
Width proto: width deut.	1.1	1.0
Greatest width: total length	3.0	4.0

Pyxinoides bolitoides sp. nov.

(Pl. 22 Figs. 5, 6.)

In addition to *Ceph. communis*, an extracellular gregarine is commonly found in *Bal. crenatus*, *Bal. cariosus*, and *Bal. glandula*. This gregarine belongs to the genus *Pyxinoides* because of the type of epimerite but differs from *Pyxinoides balani* and *Pyx. chthamali* and so a new species, *Pyxinoides bolitoides* is proposed.

The mature sporont of *Pyx. bolitoides* reaches a length of 101 μ. The longest association seen was 208 μ. The primite is usually as long or longer than the satellite. Occasionally two satellites are attached to the primite. The protomerite of the satellite fits into the deutomerite of the primite. The ratio of the length of the protomerite to the total length of the sporont is as 1 is to 2.4, the ratio of the width of the protomerite to the width of the deutomerite as 1 is to 1.1, and the ratio of the greatest width to the total length is as 1 is to 2.2. In the attached cephalont, the protomerite is often much longer than in the unattached. Apparently the stretched out protomerite tends to shorten when the epimerite pulls free from the epithelial cell. The protomerite of cephalonts and even sporonts is often wider than the deutomerite. Below are given a few typical measurements in microns.

	Cephalont		Primite	Satellite
Length protomerite	20	26	37	16
„ deutomerite	34	56	56	72
Width protomerite	20	29	37	40
„ deutomerite	20	26	38	40
Total length sporont	54	82	93	88
„ „ association			181	—
Ratio:				
Length proto: total length	2.7	3.1	2.5	5.5
Width proto: width deut.	1.0	0.9	1.0	1.0
Greatest width: total length	2.7	2.8	2.4	2.2

In the mature sporont, the endoplasm appears brown, rarely black, by transmitted light; there is no difference in the color or granules of the endoplasm of the protomerite and deutomerite. The epicyte is thin and there is a slight constriction at the septum. The deutomerite is usually slightly pointed at the posterior end. The nucleus contains one karyosome. The protomerite of *Pyx. bolitoides* is thickened at the anterior end, and often a canal which leads

from the place of attachment of the epimerite a short distance into the interior of the protomerite, can be seen.

The epimerite of *Pyx. bolitoides* is mushroom-shaped, with a crenulated border containing 12 ridges, and attached to the protomerite by an extremely short stalk. The epimerite is about 8 μ in height and 10 μ in width. Cephalonts become detached from the epithelial cell when they are 25—30 μ in length; the epimerite is almost always found on the detached forms of this size and frequently on larger forms. The epimerite is often retained on the primite following association or if the epimerite is lost, the stalk often persists as a very small knob, about 3—4 μ.

Pyx. bolitoides exhibits rapid movement in a forward direction. No bending motions have been observed.

Mixed infections with both of the other gregarines found in shore barnacles occur, as well as infections with *Pyx. bolitoides* alone.

Gregarina spissa sp. nov.

(Pl. 22 Figs. 7, 8.)

The third gregarine found in the gut of shore barnacles is not as common as either *Ceph. communis* or *Pyx. bolitoides.* It is different from any described species of gregarines in barnacles and is provisionally placed in the genus *Gregarina* as the structure of the epimerite is simple. However, it seems certain that further work on the life cycle will make it necessary to create a new genus for this form. *Gregarina spissa* is proposed for this species.

The mature sporont reaches a length of 131 μ and the longest association seen was 263 μ. The primite is usually shorter than the satellite. The deutomerite of the primite fits into the protomerite of the satellite. The ratio of the length of the protomerite to the total length is as 1 is to 2.7, the ratio of the width of the protomerite to the width of the deutomerite is as 1 is to 1.1, and the ratio of the greatest width to the total length is as 1 is to 1.6. The cephalont is also 1.6 times as long as wide. A few measurements in microns are given below (see p. 422).

The mature sporont of *Gregarina spissa* appears dark brown by transmitted light. There is no difference in color or texture of the endoplasm of the protomerite and deutomerite. The epicyte is comparatively thick, especially at the posterior end of the deutomerite, which is usually pointed. There is a deep constriction at the septum. The epicyte is thickened at the anterior end of the protomerite,

			Primite	Satellite
Length protomerite	24	37	58	44
„ deutomerite	35	58	73	88
Width protomerite	38	61	73	88
„ deutomerite	40	68	88	88
Total length sporont	59	95	131	132
„ „ association			263	—
Ratio:				
Length proto: total length	2.4	2.6	2.3	3.0
Width proto: width deut.	1.0	1.1	1.2	1.0
Greatest width: total length	1.5	1.4	1.5	1.5

especially so at the apex, giving the appearance of a plug. The nucleus contains one large karyosome.

The epimerite of *Greg. spissa* is large, spherical or subspherical, transparent and distinguished by the minute papillae covering the surface. The epimerite varies in height between 16—31 μ and in width between 31—35 μ. The epimerite is often retained by sporonts in association.

Greg. spissa has been found alone in all three species of shore carnacles and also associated with either *Ceph. communis* or *Pyx. bolitoides*. It is not found as commonly as either of the above.

Cephaloidophora magna sp. nov.

(Pl. 22 Fig. 2.)

Balanus nubilis, the large deep water barnacle of Puget Sound is quite commonly parasitized by a gregarine of the genus *Cephaloidophora*. This gregarine differs from any species heretofore described in this genus and so the name *Cephaloidophora magna* is proposed.

Sporonts of *Ceph. magna* are often found in large numbers in the gut. They are found throughout the whole length of the gut and also in the glands which open into the stomach.

The mature sporont reaches a length of 438 μ. The longest association seen was 803 μ long. The satellite is approximately the same length as the primite. In mature sporonts, the ratio of the length of the protomerite to the total length is as 1 is to 4.5, the ratio of the width of the protomerite to the width of the deutomerite is as 1 is to 1.1, and the ratio of the greatest width to the total length is as 1 is to 3.3. In immature sporonts, the length of the protomerite is greater in proportion to the total length or as 1 is

to 3.8. Actual measurements in microns of typical sporonts are given in the following table.

			Primite	Satellite
Length protomerite	46	46	73	58
„ deutomerite	160	125	292	336
Width protomerite	68	46	117	131
„ deutomerite	103	46	175	161
Total length sporont	206	171	365	394
„ „ association			759	—
Ratio:				
Length proto: total length	1 : 3.4	1 : 2.7	1 : 5	1 : 6.8
Width proto: width deut.	1 : 1.5	1 : 1.0	1 : 1.1	1 : 1.2
Greatest width: total length	1 : 2	1 : 3.7	1 : 2.0	1 : 2.4

The mature sporont of *Ceph. magna* is very striking because of its size. The granules of the endoplasm of the deutomerite are homogeneous and appear black with transmitted light, while those of the protomerite are much larger and refractive. The nucleus is large, spherical and contains numerous karyosomes, 10—13 having been counted, although 5—6 is the usual number.

The structure of the protomerite is typical of other members of the genus. A large disc, 30—57 μ in width and 25—35 μ in length is embedded in the center of the anterior end of the protomerite. About one half of the disc usually protrudes above the surface and the other half can be seen inside the protomerite. The surface of the protomerite is thickened in the region of the disc and in favorable specimens has the appearance of a band surrounding the disc. Very often one or more large deep staining granules are seen in the protomerite.

Association does not occur in this species until the sporonts are nearly mature. The satellite fits into a slight cup in the deutomerite of the primite. In most associations, the disc in the anterior end of the satellite is partially visible. Very occasionally three sporonts are in association.

Sporonts of *Ceph. magna* are very actively motile. The protomerite exhibits bending and twisting movements and easily collapses when the gregarines are in sea water on a slide. The sporonts, removed from the gut, remain active for a considerable length of time in sea water. They have been motile for as long as 24 hours when they were kept at a temperature of about 10°.

Very occasionally cysts, about 250×190 μ, have been found in the faeces. In no case did they complete their development, and

sporocysts have not been seen. Associated animals very often exhibit the typical circling motion that precedes encystment, but the formation of cysts has never been observed.

The earliest stage of the life cycle that has been seen is the intracellular cephalont before the differentiation of the protomerite and deutomerite. The development of the intracellular form closely parallels the development of *Ceph. communis* except for the fact that the cephalont of *Ceph. magna* is 40—45 μ in length before it is released from the epithelial cell.

Cephaloidophora multiplex sp. nov.

(Pl. 22 Fig. 3.)

In two rather similar species of barnacles, *Balanus balanus pugetensis* and *Balanus rostratus heteropus*, which occur below the low tide mark in the Puget Sound Region, a new species of *Cephaloidophora*, named *Ceph. multiplex*, has been found. *Ceph. multiplex* resembles both *Ceph. communis* and *Ceph. magna* in its intracellular development and in the structure of the protomerite in immature forms, and resembles *Ceph. magna* in the late appearance of syzygy, i. e. just previous to encystment. It differs from both *Ceph. communis* and *Ceph. magna* in the structure of the protomerite in the adult sporont, in the possession of a single karysome and in the type of motility.

The mature sporont of *Ceph. multiplex* reaches the length of 538 μ. The longest association seen was 864 μ. The satellite is usually longer than the primite; the length of the primites varied from 297 to 442 μ and the length of satellites from 373—518 μ. In mature sporonts, the ratio of the length of the protomerite to the total length is as 1 is to 6.1, of the width of the protomerite to the width of the deutomerite is as 1 is to 1.1, and the ratio of the greatest width to length of the sporont is as 1 is to 3.5. Below are given measurements in microns of typical sporonts.

			Primite	Satellite
Length protomerite	34	62	62	41
„ deutomerite	104	290	235	317
Width protomerite	41	83	104	104
„ deutomerite	48	83	104	104
Total length sporont	138	352	297	358
„ „ association			655	—
Ratio:				
Length proto: total length	1 : 4	1 : 5.6	1 : 4.8	1 : 8.7
Width proto: width deut.	1 : 1.2	1 : 1.0	1 : 1.0	1 : 1.0
Greatest width: total length	1 : 2.8	1 : 4.2	1 : 2.8	1 : 3.4

The mature sporont of *Ceph. multiplex* is easily distinguished from other members of this genus by the structure of the protomerite. In immature forms, both intracellular and those that have been released from the epithelial cells of the gut, there is a clear hollow crescentic area at the apex of the protomerite. Instead of rounding up into a discreet, almost spherical lens as in *Ceph. communis* and *Ceph. magna*, the anterior edge of this area becomes considerably thickened to form a clear cap at the anterior edge of the protomerite, and the distal edge flattens out to extend nearly across the protomerite to separate the anterior third from the posterior two-thirds of the protomerite. When the sporont is observed under low magnification, this plate across the protomerite is much more noticeable than the thickening at the apex.

In this form, the endoplasm appears to be the same color and consistency in the protomerite and deutomerite. There is a thick septum between these two parts and a deep constriction at the septum. The epicyte is quite thick, especially in the deutomerite, particularly at the posterior end where a tail which may extend for 10 μ beyond the deutomerite is often present. The nucleus, 34 $\times$ 34 μ, is usually in the posterior third of the deutomerite and one large karyosome is present.

Biassociation occurs in this form; the associates range in size from 297—518 μ in length, although many solitary sporonts are seen in the lower range of measurements. Syzygy does not occur until the sporonts are mature. The typical revolving motion of associates just previous to encystment has been observed. The two associates are usually not securely fastened together and the deutomerite of the primite fits into the protomerite of the satellite.

Ceph. multiplex differs from *Ceph. communis* and *Ceph. magna* in the type of motility exhibited. This form moves fairly rapidly in a straight line and rarely exhibits any bending motion which is characteristic of the other two species.

Pyxinoides pugetensis sp. nov.

(Pl. 22 Figs. 9, 10.)

In the gut of *Balanus balanus pugetensis*, in addition to *Cephaloidophora multiplex*, another gregarine was less commonly found. This gregarine is similar to that described by Tégouboff as *Pyxinoides balani* and yet differs from it to such an extent that it is proposed as a new species, *Pyxinoides pugetensis*.

The mature sporont of *Pyxinoides pugetensis* reaches the length of 242 μ. The longest satellite seen was 180 μ, and the satellite is usually shorter than the primite, although there is not as great a discrepancy in size as TRÉGOUBOFF found for *Pyx. balani*. In mature forms, the ratio of the length of the protomerite to total length is as 1 is to 2.9 and of the width of the protomerite to the width of the deutomerite is as 1 is to 1.2. The ratio of the greatest width to the length of the sporont is as 1 is to 1.7. In this form, there is only a very slight change in the comparative sizes of the protomerite and deutomerite during growth. A few typical measurements in microns are given below.

			Primite	Satellite
Length protomerite	16	92	69	35
„ deutomerite	35	112	138	117
Width protomerite	30	105	83	76
„ deutomerite	35	122	117	83
Total length sporont	51	204	207	152
„ „ association			359	—
Ratio:				
Length proto: total length	3.2	2.2	3.0	4.3
Width proto: width deut.	1.2	1.1	1.4	1.1
Greatest width: total length	1.4	1.7	1.8	1.8

In the mature sporont of *Pyx. pugetensis* the endoplasm appears very dark with transmitted light. There is no difference in color or size of granules in the protomerite and deutomerite. The deutomerite is usually pointed. The epicyte is thick and especially so at the posterior end of the deutomerite. There is a constriction of the septum between the protomerite and deutomerite. The nucleus is always visible but in the larger forms, the karyosome usually can not be seen. When visible, some individuals have one karyosome and others have several. The protomerite has a canal in the anterior end very similar in appearance to that seen in *Pyx. chthamali* by TRÉGOUBOFF and in addition a plug, 10 $\mu \times 10$ μ, is usually visible in the middle of the anterior end in those forms that have lost the epimerite. A vacuole between the protomerite and deutomerite may often be observed.

The epimerite in this form is quite similar to that of *Pyx. balani* in that it is grooved but has twenty grooves instead of sixteen. It fits down on the protomerite with no neck although in attached forms, the protomerite is often extended so that the anterior end is quite narrow. The epimerite varies in height between 7—24 μ

and in width from 11—22 μ. It is made up of two parts, an inner smaller part which occurs at the place of attachment to the protomerite and which is surrounded by the grooved second part. When the epimerite is lost, the inner part often remains as a knob 5 $\mu \times 5$ μ in size. The epimerite differs from that of *Pyx. balani* in the number of grooves, in the absence of both a mucron and a neck, and in the presence of an inner knob.

Association occurs in many cases before the epimerite is lost. The primite, however, reaches a length of 180 μ before association occurs. In a few instances, associations were seen in which two satellites were attached to the primite. The protomerite of the satellite fits into the deutomerite of the primite.

Motile forms of *Pyx. pugetensis* move in a forward direction and in addition make rapid bending motions from side to side. The forward movement is not as rapid as in *Ceph. communis* and *Ceph. magna.*

In most instances *Pyx. pugetensis* appeared as a solitary infection in *Bal. balanus pugetensis*, but occasionally both *Pyx. pugetensis* and *Ceph. multiplex* were present in the same gut. *Pyx. pugetensis* was never found in *Bal. rostratus heteropus* although this barnacle was not only closely associated with *Bal. balanus pugetensis* but was also commonly infected with *Ceph. multiplex.*

Gregarina valettei Nussbaum.

(Pl. 22 Figs. 11, 12.)

Nussbaum in 1890 described *Gregarina valettei* from the intestine of young *Pollicipes polymerus.* All his observations were made on gregarines in fixed material. The cephalont, 51 μ in length, was attached to the epithelial cell by a spherical epimerite, 7 $\mu \times 7$ μ, which was connected to the protomerite by a jagged projecting flange. From the measurements of cephalonts given in the text and measurements made of his figures, it was found that the ratio of the length of the protomerite to the total length is as 1 is to 2.2, the width of the protomerite to the width of the deutomerite is as 1 is to 1.4, and the ratio of the greatest width to the total length is as 1 is to 2.7. In the primite figured, the ratios are approximately the same except that the primite is 1.6 times as long as wide. Nussbaum does not state the maximum size of sporonts but gives two measurements of syzygies in which the primites were 60 μ and 55 μ and the satellites were 19 μ and 45 μ in length.

The nucleus, 7—10 μ, had a single karyosome. The epimerite was never found on free individuals or on those in syzygy. He

observed a small plug on the protomerite of the satellite which fitted into the deutomerite of the primite and mentioned that the primite and satellite were not always the same size. He did not see cysts or sporocysts.

Although there are several differences between the gregarine described by NUSSBAUM and the gregarine found in the gooseneck barnacle, *Mitella polymerus*, of Puget Sound, they apparently are the same gregarine. For the present, it seems advisable to consider this species as a member of the genus *Gregarina*, as in the case of *Greg. spissa*, although it seems highly probable that when the complete life cycle is known, it will be found that both these two species do not belong in the genus *Gregarina*.

The longest sporont of *Greg. valettei* seen was 182 μ and the longest association, 325 μ. The primite is almost invariably longer than the satellite, as the protomerite of the satellite fits into the deutomerite of the primite. The ratio of the length of the protomerite to the total length is as 1 is to 2.4, the ratio of the width of the protomerite to the width of the deutomerite is as 1 is to 1.2, and the ratio of the greatest width to the total length is as 1 is to 1.6. A table of a few typical measurements, in microns, is given below.

		Primite		Satellite	
		a	b	a	b
Length protomerite	37	76	34	28	21
„ deutomerite	48	104	48	117	58
Width protomerite	34	90	46	73	48
„ deutomerite	42	110	48	110	51
Total length sporont	85	180	82	145	79
„ „ association		325	161	—	—
Ratio:					
Length proto: total length	2.3	2.4	2.4	5.2	3.8
Width proto: width deut.	1.2	1.2	1.0	0.9	1.1
Greatest width: total length	2.0	1.6	1.7	1.3	1.5

The anterior end of the protomerite in all sporonts and older cephalonts shows a thickened area of the epicyte, in the center of which is a canal leading into the interior of the protomerite. This is very similar to the structure of the protomerite of *Pyx. chthamali*, as described by TRÉGOUBOFF (1912). The endoplasm of the mature sporont is quite dense and appears black by transmitted light. It is usually slightly more concentrated in the protomerite than in the deutomerite and also is often denser in the middle of the deutomerite than toward the sides. There is a slight constriction of the septum

between the protomerite and deutomerite. The spherical nucleus, 18—20 μ, is usually situated near the septum and often the single karyosome is visible in the living animal.

The epimerite in this form is spherical and measures 8—10 μ in diameter. The epimerite is very susceptible to environmental changes and usually explodes when placed in direct contact with sea water. It is made up of three parts; the smooth outer layer surrounds a papillate inner sphere, the structure of which can more easily be seen in those epimerites which have exploded. The epimerite is attached to the protomerite by a very small spherical knob (probably the projecting flange of NUSSBAUM), which can only be seen when the epimerite has been knocked off or in those forms in which the epimerite has been lost. The epimerite is often retained by gregarines in syzygy.

Greg. valettei exhibits very active bending and twisting movements of the protomerite and anterior portion of the deutomerite. The forward movement is almost as rapid as in *Ceph. communis* but does not occur very often when this gregarine is observed on a slide in sea water.

Occasionally, cysts, measuring 103—138 μ, of *Greg. valettei* have been found but the formation of sporocysts did not occur.

Greg. valettei is the only gregarine that has been found in the examination of numerous gooseneck barnacles from three islands in Puget Sound and from Seal Rock and Waaddah Island in Neah Bay. The various shore barnacles, particularly *Bal. cariosus*, closely associated with *Mitella polymerus* have never been infected with *Greg. valettei* but in many cases were heavily infected with any or all of the three gregarines found in these barnacles.

Greg. valettei is another example of host specificity in gregarines. As has been mentioned above, *Pyx. pugetensis* is found only in *Bal. balanus pugetensis*, even though this barnacle is closely associated with *Bal. rostratus heteropus*. Both of these barnacles are commonly infected with another gregarine, *Ceph. multiplex*. Host specificity of gregarines in barnacles is especially interesting because of the gregarious habit of the host.

Other Barnacles Examined.

Examination of the gut of numerous *Balanus hesperius*, for the most part on *Pecten*, has resulted in negative findings except in one barnacle. A few typical sporonts of *Ceph. communis* were found in this one instance. Failure to find a greater incidence of infection

in this species may be due to the fact that most of the barnacles examined occurred as solitary individuals or, more rarely, in small groups, in contrast to the crowded condition of most of the other barnacles examined.

Numerous *Bal. crenatus*, found singly or in small numbers on *Pecten* and other molluscs in deep water, have also been negative for gregarines, in contrast to the heavy infection of *Bal. crenatus* found on the shore. This would seem to bear out the supposition that the chances of infection are comparatively slight in those cases in which the barnacles occur in isolated habitats.

A large number of *Lepas hillii* and *Lepas antifera* from several different localities have been examined from time to time, and no gregarines have ever been found. No explanation for the failure to find gregarines in these barnacles is apparent.

In addition a few whale barnacles, *Corunula diadema*, collected at the whaling station, Rose Harbor, British Columbia, were examined for gregarines. No parasites were found.

Summary.

1. A large number of barnacles of Puget Sound and adjacent waters have been examined for gregarines.

2. *Ceph. communis* MAWRODIADI has been found commonly in *Bal. crenatus*, *Bal. glandula*, and *Bal. cariosus*.

3. *Greg. valettei* NUSSBAUM of *Mitella polymerus* has been redescribed.

4. Five new species of gregarines, belonging to the genera *Cephaloidophora*, *Pyxinoides*, and *Gregarina* have been described from the following barnacles: *Bal. crenatus*, *Bal. glandula*, *Bal. cariosus*, *Bal. nubilis*, *Bal. balanus pugetensis*, and *Bal. rostratus heteropus*.

5. The following barnacles were negative for gregarines: *Bal. hesperius*, *Lepas hillii*, *Lepas antifera*, and *Corunula diadema*.

6. Two interesting examples of host-specificity are recorded.

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Explanation of plate.

Plate 22.

(All drawings from living gregarines except Figs. 5 and 9.)

Fig. 1. *Ceph. communis.* × 560.

Fig. 2. *Ceph. magna.* × 135.

Fig. 3. *Ceph. multiplex.* × 135.

Fig. 4. *Ceph. communis* from the gut of *Balanus cariosus.* × 260.

Fig. 5. Cephalont of *Pyx. bolitoides* from section of gut of *Bal. glandula.* × 560.

Fig. 6. *Pyx. bolitoides.* × 320.

Fig. 7. Cephalont of *Greg. spissa.* × 320.

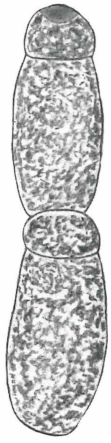
Fig. 8. *Greg. spissa.* × 135.

Fig. 9. Cephalont of *Pyx. pugetensis* from section of gut of *Bal. balanus pugetensis.* × 320.

Fig. 10. *Pyx. pugetensis.* × 135.

Fig. 11. Cephalont of *Greg. valettei.* × 320.

Fig. 12. *Greg. valettei.* × 320.



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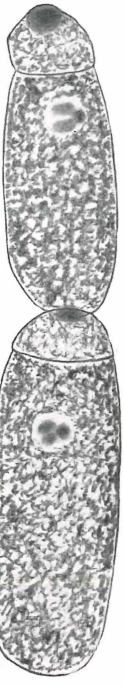

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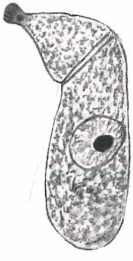

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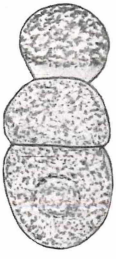

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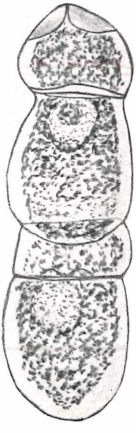

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Henry.

VERLAG VON GUSTAV FISCHER IN JENA.