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Studies on the Cephaline Gregarines from Some Barnacles in Japan¹⁾

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(With 1 Text-figure, 3 Tables and 2 Plates)

The gregarines from the Cirripedia have been studied by many workers in Europe and America. Though the studies of the Japanese Cirripedia have been well made, the gregarines from them have been treated in Japan only by H. Hoshide (1951) up to the present. Of the gregarines reported from barnacles, 5 species belonging to the genus *Pyxinooides* are known as follows: *Pyxinooides balani* Tregouboff 1912, *P. cthamali* (Léger and Duboscq) Tregouboff 1912, *P. bolitooides* Henry 1938, *P. pugetensis* Henry 1938, and *P. fujitsubo* Hoshide 1951.

In this paper 3 new species of *Pyxinooides* from Japanese barnacles, with special reference to their morphological and ecological characters, are described.

I am deeply grateful to Prof. M. Yamada under whose direction this work has been carried out and also to Dr. Sh. F. Sakagami for his valuable suggestions and criticisms.

Material and Methods

The barnacles were taken from April, 1967 to August, 1968 at the coasts of Hikari, Obatake, Hofu (Yamaguchi Prefecture), Oshoro, Asari, Akkeshi (Hokkaido).

The barnacles being torn off from rocks with a small shovel were brought into the laboratory and were kept alive for about 10 days in an ice-box as almost fresh as when they were captured.

The alimentary tract of the host was cut off from the shell with a pair of scissors and then put sea water into a Petri-dish. In the sea water I took off carefully the sperm sacks from the gut. If the sperm sacks were failed to be removed off completely without being broken, it was difficult to observe the parasites because the water become cloudy with the sperm. The naked gut was brought into drops of seawater on a clean slide glass and cut open with fine needles.

The parasites came out freely into the water, but sometimes they stuck to the wall of the intestine with their epimerites. In such case, the intestine was left as it was. After a time when the tissue of intestine was going to collapse, the gregarines were easily got with the epimerite in the complete form.

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The parasites were best studied alive and sea water was found to be the best medium for observing the living gregarines. But both smeared and sectioned preparations were often used to observe the contains of nucleus, ecto- and endoplasm, and the attaching state of parasites in the host's intestine. Smears of gut contents and material for sectioning were fixed in various media, of which both Bouin's and Schaudinn's solutions proved most suitable. Most of these preparations were stained with Heidenheim's or Delafield's haematoxylin, and some with methylblue or safranin.

In this paper, the following abbreviations are used:

TL=Total length of sporadin (or primate)

LP=Length of protomerite (or primate protomerite)

LD=Length of deutomerite

WP=Width of protomerite (or primate protomerite)

WD=Width of deutomerite

tl=Total length of satellite

lp=Length of protomerite of satellite

ld=Length of deutomerite of satellite

wp=Width of protomerite of satellite

wd=Width of deutomerite of satellite

Pyxinoides kurofuji n. sp.

(Fig. 1. A, B, C; Pl. XI. 1-9)

Host: *Tetraclita squamosa japonica* Pilsbry.

Habitat: Intestine.

Locality: Hikari (Yamaguchi prefecture).

The host is a common barnacle attached on rocks near a low tide mark on the coast of the Inland Sea of Seto. Barnacles were collected from various localities, Hikari, Hofu and Obatake in Yamaguchi prefecture, but infected specimens were obtained on the naked rocks confronting the open sea at Murozumi Bay in Hikari city. From the same species of barnacle at this place, no gregarine was found by H. Hoshide in 1951, while this time I found that they were parasitized by gregarine of the genus *Pyxinoides*.

About 20 percent of more than 200 individuals of the barnacle examined were found to be infected, but the infection was not so heavy, generally from two to six per one host. On very rare occasions the intestine of the host was heavily affected with 150 or more associations.

Sporadins: The mature sporadins are biassociative. The body is ovoidal to elongate ovoidal in shape. The sporadin reaches a length of 250μ and the longest association observed is 440μ .

The average size of the body is as follows; all dimensions are expressed in microns:

TL 210, LP 66, LD 147, WP 80, WD 136, tl 171, lp 46, ld 125, wp 78, wd 119.

Ratio; LP: TL=1:3.3, WP:WD=1:1.7, lp: tl=1:3.7, wp:wd=1:1.5.

(Primate): The primate is usually larger than the satellite. The protomerite is hemispherical and is always a little wider than long. Its anterior end is low conical with a small transparent cylindrical projection where the epimerite attaches.

The septum between protomerite and deutomerite is distinct but the constriction is not so clear. The deutomerite sometimes projects, in a broadly rounded cone-shape, into the base of protomerite.

The deutomerite is almost ovoidal or spherical. It widens gradually from the septum to the posterior portion of the body where it is widest. The posterior end is somewhat concaved to receive the anterior end of the satellite.

(Satellite): The interlocking device between primite and satellite is well developed. There is a small pit at the top of protomerite, into which the posterior end of primite is inserted and then the two individuals combine closely with each other. The satellite is ovoidal and is shorter than the primite. The protomerite is disc-shaped, convex at the top and concave at the bottom. The deutomerite is rather spherical and generally rounded at the posterior end, but some individuals have a small projecting cone here.

Table 1.

Total length of association	375	345	370	385	390
Primite					
TL	210	200	190	215	230
LP	60	65	55	70	70
LD	150	135	135	145	160
WP	90	75	65	85	90
WD	150	125	110	130	135
Ratio					
LP:TL	1:3.5	1:3.1	1:3.5	1:3.1	1:3.3
WP:WD	1:1.7	1:1.7	1:1.7	1:1.5	1:1.5
Satellite					
tl	165	145	180	170	160
lp	40	40	45	50	40
ld	125	105	135	120	120
wp	85	75	70	75	75
wd	145	125	100	105	110
Ratio					
lp:tl	1:4.1	1:3.5	1:3.2	1:3.4	1:4.0
wp:wd	1:1.7	1:1.7	1:1.4	1:1.4	1:1.5

All dimensions are expressed in microns.

The epimerite of *P. kurofuji* is often retained on the primite composing association. It is flower-shaped, and formed of two parts: a small cup-like base and a spherical head. The head, measuring about 10μ in diameter, is composed of thin membranes covering the base. Fine longitudinal grooves, 15-16 by count, are seen on its surface. These grooves become clearly discernible when the individual is fixed and dyed with haematoxylin. The epimerite is very frail, then

almost all the gregarines detached from the wall of host's gut, have a broken head with opened petals. These petals attach to the base cup-like stalk.

In the mature sporadins, the endoplasm is dense and light brown in color, containing minute, homogeneous granules. The granules of the endoplasm of the protomerite are a little smaller than those of the deutomerite. The epicyte is very thick and stout, especially at anterior one third part of the protomerite, where it measures about 25μ in thickness. The border margin between the anterior thick and the posterior thin epicytes shows a wavy-like line. Near around the septum the epicyte is also thick, 10μ in thickness, and the endoplasm pressed here appears a light brown band around the anterior part of the deutomerite.

Nucleus is spherical and measures from 35μ to 40μ in diameter. It situates generally in the center of deutomerite and contains one spherical nucleolus, measuring about 15μ in diameter.

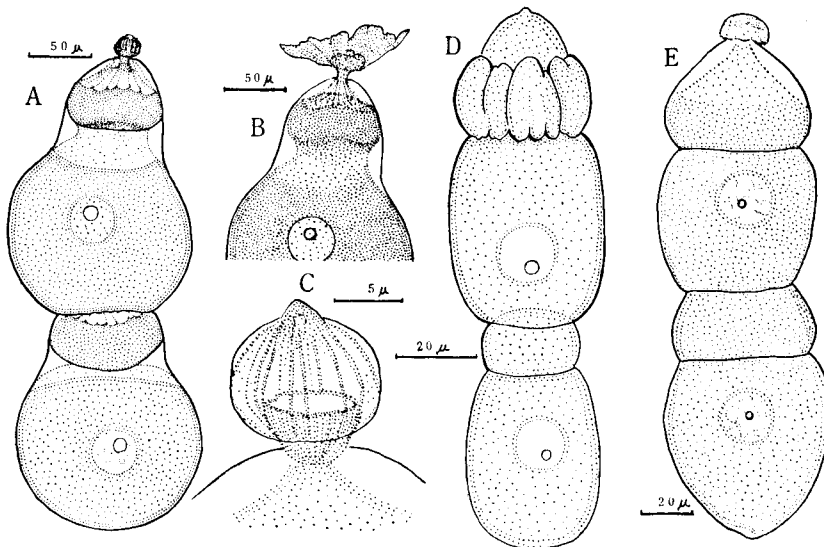


Fig. 1. A. Mature association of *Pyxinoïdes kurofuji* n. sp. B. Anterior part of body with a broken epimerite of the same species. C. Enlarged epimerite of the same species. D. Mature association of *P. japonicus* n. sp.; the protomerite of primitive is shown. E. Mature association of *P. oshoroensis* n. sp.

Cephalins: Cephalins detached from the epithelial cell, contract and become spherical. In sections of the host's intestine, they are found to be elongate ovoidal in shape. The epimerite is inserted within the cell of the host and the anterior part of the body is sometimes much extended in a slender cone-shape, in which several longitudinal stripes stained deeply with dyes are visible.

Remarks: Although the cyst and spores were not observed this species may clearly belong to the genus *Pyxinooides* for its characters of sporadins and the shape of the epimerite.

Among the members of the genus *Pyxinooides*, it resemble *P. pugetensis* and *P. cthamali* in the size and ratio of the body, but it differs from *P. cthamali* in the shape of the epimerite, in that the later has a typical one with a long style. This species is easily distinguished from *P. pugetensis* by the difference of the shape of body and the character of epimerite.

***Pyxinooides japonicus* n. sp.**

(Fig. 1. D; Pl. XII. 1-14)

Host: *Cthamalus challengeri* Hoek.

Habitat: Intestine.

Locality: Oshoro, Asari and Akkeshi (Hokkaido), Hikari and Hofu (Yamaguchi prefecture).

The host is a small rock barnacle, which stuck gregariously on to tide-rocks. I found these barnacles to be infected with small species of gregarine, which belongs to the genus *Pyxinooides* because of the type of epimerite.

During the winter of 1967, parasitism was found to occur in about 20% of the barnacles examined in Yamaguchi prefecture, but in 38% of the barnacles collected at Oshoro in Hokkaido, from April to June, 1968.

Cephalins: The earliest stages of this species observed were the young cephalins, measured 18-20 μ long and 10-12 μ wide. These are provided with a comparatively large, ovoidal epimerite, 5-7 μ in length, buried in the epithelial cell of the host's gut.

For example, measurements of a fairly large cephalins are, in microns as follows: TL 42, LP 15, LD 28, WP 17, WD 25, Epimerite 5 \times 4, Nucleus diameter 15.

The protomerite is conical, but it occasionally shows a complex figure. Its anterior one third is conical and the posterior two thirds are divided irregularly into several lobes, the former part projects into the latter lobes, which surround the base of the projecting cone. The irregular margin of the lobes hangs posteriorly over the anterior parts of deutomerite, but the septum between protomerite and deutomerite is seen clearly.

The epimerite of this species is mushroom-shaped, and it is divided into two parts: an ovoidal or spherical crown and a stalk. The border of the crown is crenellated at its under half with fine ridges. The crown measures 5 \times 6 μ in size. Cephalins become detached from the host's epithelial cell when they are 20-40 μ in length, the epimerite is usually found attaching to the protomerite with stalk. In some specimens, the part of the crown is already lost; the stalk only remains as a comparatively long knob, 6 \times 3 μ in size.

The epimerite is usually lost before the association occurs.

Sporadins: The sporadins are biassociative and ovoidal to elongate ovoidal,

On rare occasions, however, they associate in three; two satellites together attach to the posterior end of one primate. The mature sporadin reaches a length of 140μ and the longest association seen was 250μ .

The average size of the body is as follows; all dimensions expressed in microns:

TL 73, LP 33, LD 38, WP 40, WD 48, tl 83, lp 13, ld 70, wp 38, wd 48.

Ratio; LP:TL=1:2.2, WP:DW=1:1.2, lp:tl=1:6.6, wp:wd=1:1.3.

(Primate): The protomerite is conical or hemispherical. In living specimens, the posterior two-thirds of protomerite sometimes swell and irregular lobes appear on the surface of the part, as described above in the case of cephalins. There is a deep constriction at the septum.

The deutomerite is short cylindrical or rather spherical in shape. The posterior end of deutomerite is generally concaved upward, as for the anterior end of satellite fits there.

Table 2.

Total length of association	166	144	143	169	143
Primate					
TL	82	62	64	82	64
LP	40	30	30	32	30
LD	42	32	32	49	32
WP	49	40	37	37	37
WD	64	44	42	44	42
Ratio					
LP:TL	1:2.1	1:2.1	1:2.2	1:2.5	1:2.2
WP:WD	1:1.3	1:1.1	1:1.1	1:1.2	1:1.1
Satellite					
tl	84	82	79	87	79
lp	15	15	12	12	12
ld	69	67	67	74	67
wp	42	35	37	32	37
wd	72	37	42	42	42
Ratio					
lp:tl	1:5.7	1:5.5	1:6.4	1:7.0	1:6.4
wp:wd	1:1.7	1:1.1	1:1.1	1:1.3	1:1.1

All dimensions are expressed in microns.

(Satellite): The satellite is elongate ovoidal, and it is generally longer than the primate, although the sizes of both primate and satellite does not make much difference. Associations whose primites are longer than the satellites are rarely seen. The protomerite is usually lower than that of primate and is somewhat flattened at the top and bottom. The deutomerite is ovoidal and the posterior end ending in a well rounded or sometimes in a pointed extremity.

In the mature sproadins of this species the endoplasm appears light brown

and it is not so dense with coarse granules. There is no difference in color and size of granules in the protomerite and deutomerite. The epicyte is very thick over the whole body, and especially so at the anterior region of the protomerite, and often a fine canal from the place of attachment of the epimerite into the inner part of protomerite can be observed.

The nucleus is spherical, 13–15 μ in diameter, and contains one spherical nucleolus, 5 μ in diameter, which is well dyed with safranin and haematoxylin.

Remarks: As this species has a typical type of epimerite, it may belong to the genus *Pyxinoides*. Among the members of this genus it resembles *P. bolitoides* in the size of the body but this is easily separated from the latter because it has a unique protomerite. I propose this species as a new member of *Pyxinoides*, *P. japonicus* n. sp.

***Pyxinoides oshoroensis* n. sp.**

(Fig. 1. E; Pl. XII. 15–18)

Host: *Balanus cariosus* Pallas

Habitat: Intestine.

Locality: Oshoro, Asari and Akkeshi (Hokkaido).

The host, *Balanus cariosus* is a kind of rock barnacles, which is found commonly at the coasts in Hokkaido. The habitable places for this barnacle usually face an open sea where rough waves dash against the rocks. From the spring to summer in 1968, I collected the barnacles and observed the gregarines in their intestines. Almost all barnacles examined were found to be parasitized by this species of the genus *Pyxinoides*. Always no other species of gregarines was seen in the same host. Although this gregarine may be parasitic on an almost whole intestine, it is mainly found to live upon its posterior region.

Sporadins: The mature sporadins are biassociative and ovoidal in shape.

The average size of sporadins is as follows; all dimensions are expressed in microns:

TL 109, LP 42, LD 67, WP 63, WD 77, tl 85, lp 28, ld 57, wp 58, wd 66.

Ratio; LP:TL=1:2.6, WP:WD=1:1.2, lp:tl=1:3.0, wp:wd=1:1.1.

The longest association seen was 222 μ .

The protomerite of primate is hemispherical, widest near the septum. In the attached individuals the protomerite is often much stretched out and is longer than in unattached ones. The protomerite of the free specimens becomes shorter. There is a distinct constriction between protomerite and deutomerite. The deutomerite is rather globular when the parasites mature. Sometimes at the end of the deutomerite of satellite or cephalins, a small conical projection is seen.

The primate is usually longer than the satellite.

In the mature sporadins, the endoplasm appears light brown by the transmitted light. There is no difference in the color or character of granules in the endoplasm of the protomerite and deutomerite. But the endoplasms of the

Table 3.

Total length of association	194	184	167	191	222
Primate					
TL	116	106	96	103	121
LP	38	45	38	38	50
LD	78	61	58	65	71
WP	55	63	55	73	63
WD	66	76	66	93	81
Ratio					
LP:TL	1:3.1	1:2.3	1:3.1	1:2.7	1:2.4
WP:WD	1:1.2	1:1.2	1:1.2	1:1.2	1:1.3
Satellite					
tl	78	78	71	88	101
lp	23	30	20	33	35
ld	55	48	51	55	66
wp	53	63	40	58	66
wd	53	73	45	66	78
Ratio					
lp:tl	1:3.4	1:2.6	1:3.5	1:2.6	1:2.9
wp:wd	1:1.0	1:1.2	1:1.1	1:1.1	1:1.2

All dimensions are expressed in microns.

primate and satellite have different characters each other; the former is much denser and dyed deeper than the latter with haematoxylin.

The epicyte is quite thick, especially in the anterior portion of protomerite. In the center of this thickened area, a canal leads from the place of the epimerite into the anterior of the protomerite.

The epimerite is swollen, mushroom-shaped. It is composed of two parts: the basal cup-like body and the thin membranous part covering the cup. The border of the cup is crenellated with about 16 ridges. The epimerite often retained on the primate composing association. When the epimerite is lost, a transparent small knob is seen on the top of the protomerite, which is considered as a stalk, setting the epimerite on the protomerite. The nucleus is large and spherical, 20–28 μ in diameter, and it contains one spherical nucleolus.

Remarks: From *Balanus cariosus* obtained in Europe and North America, two species of gregarine have been known till now, i.e., *Cephaloidophora communis* Mawrodiadi and *Pyxinoides bolitoides* Henry.

The present species from the same species of barnacles clearly belongs to the genus *Pyxinoides* by the shape of the epimerite.

This species resembles *P. bolitoides* in length of the sporadins but differs in the following points:

- 1) As for the mature sporadins, in the width of the body, the former is

almost twice as wide as the latter. 2) In the shape of the epimerite, the former is swollen and the latter flat, the number of ridges on the border of the crown is 16 in the former, whereas 12 in the latter.

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Explanation of Plates XI-XII**Plate XI**

Figs. 1-9: *Pyxinoides kurofuji* n. sp.

1. Mature association: sporadins are elongate ovoidal.
2. Another mature association: sporadins are ovoidal.
3. Association fixed with Bouin's solution and stained with Delafield's haematoxylin.
4. A spherical trophozoite which has lost its epimerite.
5. An elongate cephalin sticking to the host's gut, the epimerite is buried in the epithelial cell.
6. A young cephalin in the host's intestine.
7. Epimerite, attaching to the anterior end of protomerite.
8. Broken epimerite; membranous open petal attached to the basal cup is seen.
9. Spherical nucleus containing one nucleolus.

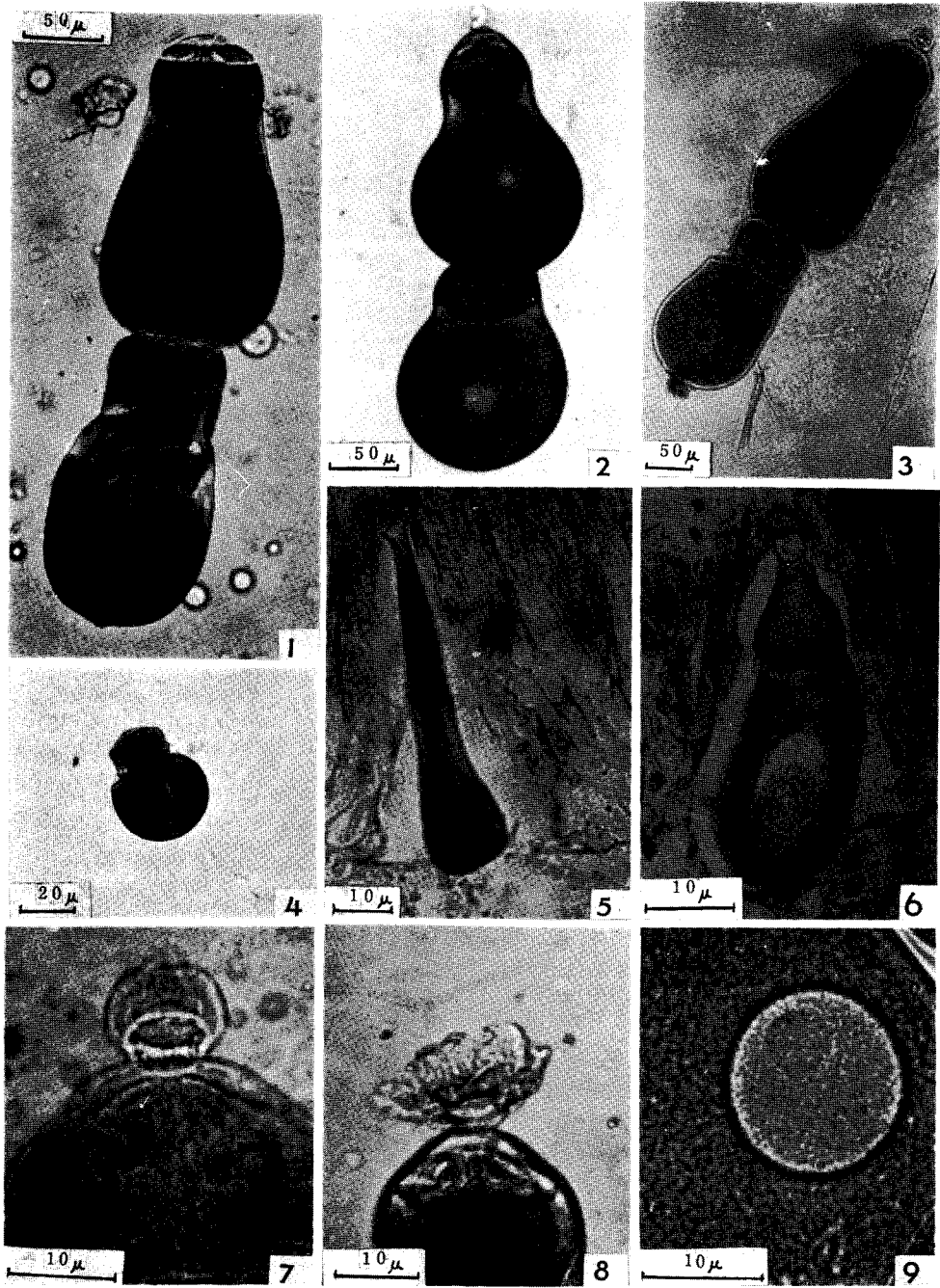
Plate XII

Figs. 10-14: *Pyxinoides japonicus* n. sp.

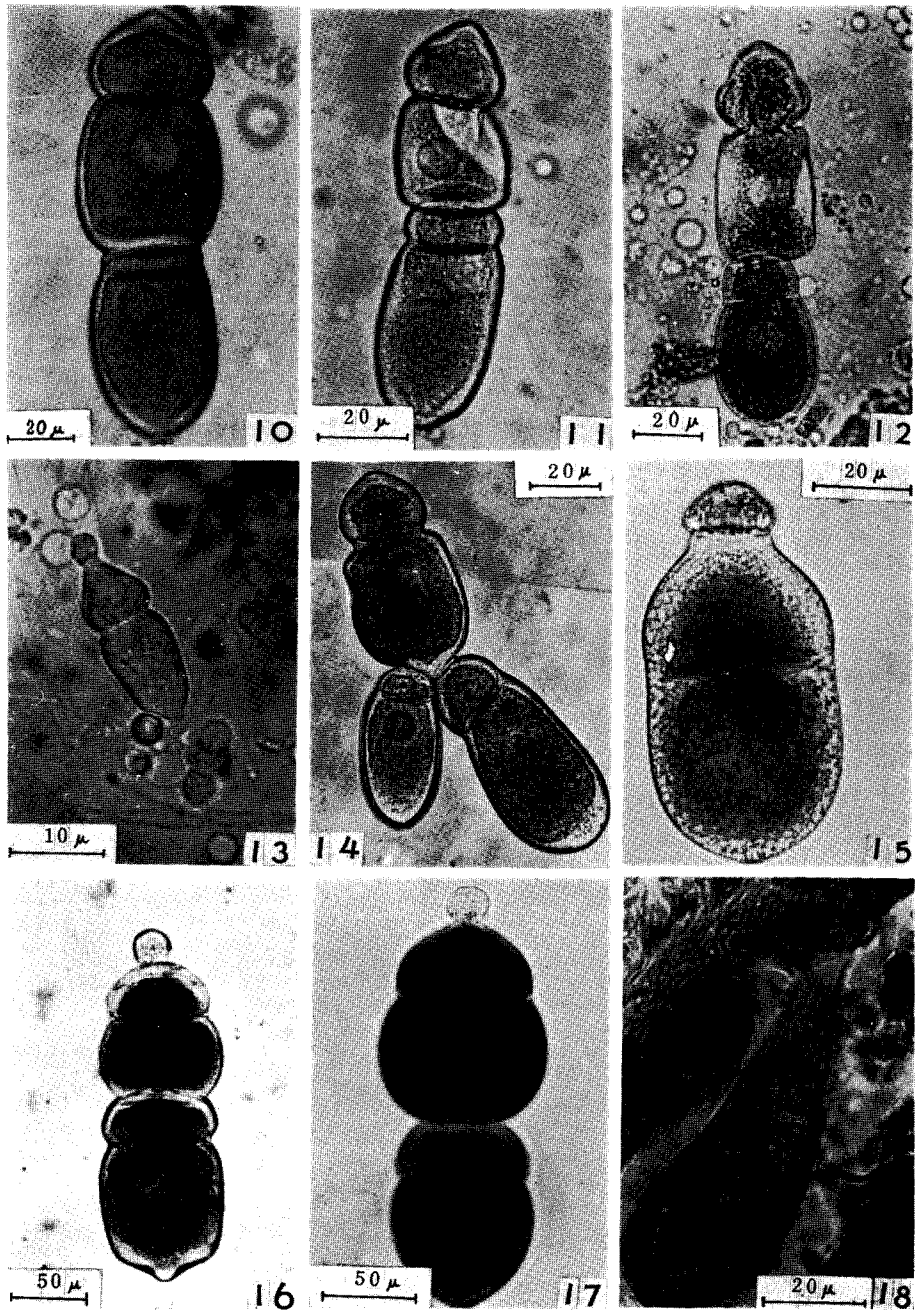
10. Mature association.
11. Another mature association.
12. An association: the protomerite of primite appears a complex figure.
13. Small cephalin with epimerite.
14. The association: two satellites attach to one primite.

Figs. 15-18: *Pyxinoides oshoroensis* n. sp.

15. A cephalin with epimerite.
16. Mature association retaining the epimerite.
17. Another association stained with Delafield's haematoxylin.
18. A cephalin sticking to the host's epithelial cell which is inserted into epimerite.



K. Hoshide : Cephaline Gregarines from Barnacles



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