# Recent researches

ON THE

# Choanoflagellata (Craspedomonadines)

## (Fresh-water and marine)

WITH DESCRIPTION OF NEW GENERA AND SPECIES

BY

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With the evidence which the writer has obtained, and hereafter adduces, in support of the axopodal constitution of the choanoflagellate collar, the intimate relationship which LAMEERE and DE SAEDELEER believe to exist between the *Choanoflagellata* and the *Helioza* (and in a less degree, the *Foraminifera* and *Amoebae*, and, still more distantly, the *Radiolaria*) seems to be established.

The choanoflagellate cell is at once extremely simple and complex. It is a single, mono-nucleate cell with a single flagellum. Its complexity lies chiefly in the constitution and functioning of its characteristic appendage — the collar, and in its extraordinary secretory powers.

From a combined morphological and physiological point of view, in no other order of *Protozoa* is there found such variety of secreted tegumentary structures, ranging as these do from a hyaline, *Bacteria*-inhibiting jelly, through a series of firmer protective structures, the products of secretions of a more or less chitinous nature (here provisionally and collectively named the pseudo-lorica) up to the definitely siliceous structure — the true lorica.

Morphologically the *Choanoflagellata* are comparable in importance with the *Foraminifera* and *Radiolaria*, and physiologically their evolutionary status is considerably higher. In fact LAMEERE (1) considers them to be the direct ancestors of the Metazoa. The reasons for these conclusions will appear in what follows.

(1) LAMEERE, 1928, Précis de Zoologie. Les Protozoaires (avec 158 figures). In : *Recueil Inst. Zool. Torley Rousseau*, Univ. Bruxelles.

Food inception. — The problem of food inception in the species *Codonosiga botrytis* was investigated in January 1922, the conclusions reached being practically identical with those obtained by De Saede-leer in 1927 (1). These conclusions are at variance with those of Ehrlich, Burck, Lapage and others, but are in part agreement with that of Stokes (8). Food ingestion in a number of other species has been observed, and it was concluded that, in most cases, the process is merely a modification of that presented by other protomastiginous Flagellates (fig. 31) in which a surface protrusion of endo-sarcodic plasm acts as an engulfing pseudopode which, together with the engulfed prey, is withdrawn into the sarcode, where digestion of the prey follows. The rhythmic and alternating productions of two such pseudopodes, — having, perhaps, special characteristics, — on opposite sides of the cell is peculiar to the illoricate species of Choanoflagellates. The production of a basal reservoir-vacuole, which may be of considerable size, is also probably peculiar to these primitive species.

This basal vacuole is formed by the coalescence of a succession of food-engulfing, pseudopodically-produced vacuoles, which, after entry (with or without captured prey) within the endosarc, have been conducted by a kind of sarcodic peristalsis direct to this basal region (De Sae-deleer has noted this, with other details, exactly, and agrees with the present writer in expressing astonishment that Lapage and his predecessors overlooked the obvious significance of this basal vacuole).

The writer and De Saedeleer agree that the loge or mucous envelope, which is the sole, specific protective investment in the *Codonosigidae*, plays no part whatsoever in the actual ingestion of food.

The primary function of the flagellum, when present in the mature choanoflagellate cell is, by creating currents of a suitable character in the surrounding water, to direct potential food against the outer surface of the collar. The function of the collar is, by means of the sticky plasm upon its surface, to retain the prey and conduct it, by the direct downward movement of this plasm, within reach of the prehensile pseudopode or the simple endo-sarcodic upheaval destined or produced to engulf it.

Food ingestion in the *Choanoflagellata* is, therefore, accomplished in the majority of species by the inter-action of the flagellum, the collar with its adhesive surface-mucus, and a more or less evident engulfing pseudopode.

Food consists, probably, exclusively of *Bacteria*. In the case of the new genera *Stephanoeca* and *Diaphanoeca* in which (a) the flagellum is

apparently functionless, (b) amorphous food masses are invariably present in the basal region of the sarcode, (c) visible food inception has never been observed, and (d) the lorica completely surrounds the cell, collar, and flagellum, and seems to be actually designed to prevent the free access of ordinary food-organisms to the collar, the writer is of opinion that ultra-microscopic *Bacteria* are ingested by the direct agency of plasmic currents, which conceivably originate near the base of the cell, and the main trend of which is — as De Saedeleer suggests is the case in all genera — up the inside and down the outside of the collar; this downward current, of course, conveying the invisible food.

Visible food inception in several species of *Salpingoecidae* is effected by the extrusion of a linguiform pseudopode at any point around the immediate exterior of the base of the collar, the selected prey being always engulfed on the outside of the collar and drawn therefrom into the sarcode.

In other cases, both visible and invisible food is ingested probably by the sole agency of a more or less definite system of collar currents : no sarcodic extrusions being produced.

The writer has frequently seen a *Bacterium*, obviously immersed in collar-plasm and at a considerable height up the collar, glide direct into the sarcode at the cell's anterior without producing any appreciable change in the contour of the neck of the cell. And the writer would here remark, in defence of his provocative but considered opinion relative to ultra-microscopic food, that he knows of no logical or biological reason why the potential food organisms of any of the *Protista* should cease to exist at the precise limit of optically aided vision.

The collar. — Observations on a marine species of *Pteridomonas* (fig. 28) in March 1926 led to the supposition that the wreath of filipodes in this genus might be homologous with the choanoflagellate collar, and therefore that the collar is a modified polyfilipodal structure.

De Saedeleer finds a closer homology, in the case of *Codonosiga*, with the cyrtophorine *Heliozoa* in which the axopodes correspond to the collar and the lobopodes to the prehensile pseudopodes.

But an even more striking homology seems to be provided by the loricated organism, which De Saedeleer considers may be a species of *Mikrogromia* (see fig. 30), and which was found by the writer in April 1926 in a fresh-water gathering sent to him from Padstow. In its general appearance it strongly resembled one of the smaller *Salpingoecidae*, the lorica being flask-shaped, the cell posteriorly-globular, with a neck extending up and beyond the neck of the lorica and bearing ten

or more non-suctorial, radiating filipodes. Moreover, it soon became evident that the latter were sticky along their whole length, since *Bacteria* were seen to adhere to them at different heights. And to clinch the resemblance, several of these captured *Bacteria* were seen to be engulfed by linguiform pseudopodes emitted near the bases of the filipodes concerned, and the food conveyed to the base of the cell in a vacuole which there coalesced with a larger vacuole. The only detail wanting to complete the absolute identity of this organism with a Choanoflagellate in which a number of sticky and independent filipodes had replaced the collar, was the flagellum.

But the writer discovered in 1924 a marine Choanoflagellate which has completely dispensed with its flagellum, except for the locomotory and temporary use of the young cell (see later description of *Choanoeca*), and so it is submitted that we have here, apparently, absolute confirmation, not only of the near relationship of the *Choanoflagellata* with the *Heliozoa* and the *Allogromiidae* (which are related to the Foraminifera), but of the polyfilipodal (or polyaxopodal) structure of the collar.

The following quotation from Saville KENT partially bears out the above conclusions and will fitly serve to introduce the subject next discussed by the present writer : " Having regard to the circulatory currents or cyclosis manifested by the sarcode substance of which it (the collar) is composed, there can scarcely be room for doubt that this structure finds its precise homologue in the pseudopodia of the Foraminiferous Rhizopods in which a similar circulation of the constituent sarcode is exhibited. The collar may in fact be most appropriately compared in this connection to a funnel-shaped aggregation of the single anteriorly-protruded pseudopodic fascicle of some Monothalamous Foraminifer such as *Lagena* or *Miliola*... The central flagellum secures the Choano : group from being placed among or closely adjacent to such typical Rhizopods ".

FREE SURFACE-PLASM OF COLLAR. — DE SAEDELEER postulates a definite plasmic current, of sarcodic origin, flowing up the inner and continuing down the outer collar surface ; and, like LAPAGE, he attributes the observed upward movement of particles adhering to the collar to the stronger upwardly-directed water-currents set up by the flagellum. This, however, does not explain the fact that particles are seen in upward movement when the flagellum is motionless. And the writer, therefore, submits the following alternative hypothesis :

Assuming that the collar is a structure composed of a number of extremely flattened axopodes with their edges either coalescing or touching,

it is conceivable that each of these component axopodes has retained its basic individuality, and thus may be the seat of separate plasmic movements varying in axial direction under varying stimuli.

The writer conceives the upper two-thirds of the collar to be composed of a wreath of long slender axopodes (filipodes), while the lower third is made up of an additional exterior wreath of shorter axopodes or lobopodes.

The upper, thinner portion of the collar, apart from its primary duty of capturing prey, performs two other tasks of equal importance viz, the overcoming, when possible, of the flagellar currents which oppose the downward movement of its plasm when burdened with potential food ; and the conveying upwards of particles, rejected as food, to the collar mouth. The duties of the lower portion of the collar may also be three-fold : (1). The actual selecting of suitable food ; (2) the engulfing by its own plasm of such food and conveying it, without the intervention of engulfing pseudopodes, direct into the sarcode ; (3) the rejecting of unsuitable food, either by applying the necessary stimulus to the descending current which brought it, in order to bring about a reversal of that current and so have the distasteful particle removed ; or itself conducting the particle to the nearest upward-flowing current to the same end.

All this is, of course, pure speculation, but it is based on observed facts which, it is claimed, it more or less adequately explains.

THE DOUBLE COLLAR. — To account for the well-developed supplementary collar in *Dicraspedella perplexa* (fig. 8) we have only to extend the above hypothesis and assume that here the supposed exterior wreath of lobopodes of the ancestral form is abnormally long and correspondingly slender, and has not merged with the main collar the whole of its length. In this genus prehensile pseudopodes (as in *Codonosiga*, but apparently non-functional for food inception) also are present, and DE SAEDELEER considers these to be evolved ancestral lobopodes of the *Cyrtophora*-group.

With regard to the reality of the supplementary collar, which has been strongly questioned by DE SAEDELEER in the case of practically all the species described by LEMMERMAN, the photographs and observational details submitted to him by the writer have convinced him that in *Dicraspedella* this extra collar is undoubtedly the structure it appears to be, and is not, for instance, an abnormally extended mucous envelope or loge.

The writer has also detected a similar second collar in *Salpingoeca*

*inquillata* or some species closely resembling it. This collar is much smaller than that of *Dicraspedella*, but its position is the same in both species — that is, the upper limit of the lower third of the main collar ; and its presence is usually indicated by a conspicuous zone formed of *Bacteria*, etc., which have accumulated in the trough which it forms with the main collar.

A fairly convincing photograph of this really difficult object has been obtained, and its realness has been confirmed in a considerable number of individuals. The writer, moreover, believes that it will be found in other species in which the above-mentioned bacterial zone is seen crossing the collar.

Food inception in *Dicraspedella* has been observed on only one occasion. The prey, a comparatively large *Bacterium*, was one of several which had accumulated in the above-mentioned trough, through the bottom of which it appeared to fall, but which was actually being conducted, by the combined movements of the plasm on the two collar surfaces, between the layer forming the lower portion of the secondary collar and that part of the main collar below it, direct into the sarcode, without the intervention of either of the two prehensile pseudopodes seen in action at the time. This observation proves, therefore, that there is no actual coalescence of the two collars where they meet, and it also appears to substantiate the conception that the two collars are derived from two distinct axopodal whorls, and that the prehensile pseudopodes are the remains of ancestral lobopodes.

The conclusion arrived at in regard to the secondary collar is that its function is a negative one — in fact, that it is a useless residual structure like the human appendix.

EXCESSIVE PRODUCTION OF COLLAR-PLASM. — In many species, in which the flagellum is abnormally active, *Bacteria*, etc. which have been rejected as food, are conducted to the mouth of the collar, where, instead of being whirled away individually by the lashing flagellum, they are seen to glide jerkily but slowly from the collar lip, and remain nearby in company with other particles, the whole collection being imbedded in a plasmic matrix, since it vibrates *en masse* with the violent movements of the flagellum.

The reason for the outpouring of this excess of plasm is obscure, unless it be one of Nature's humbler means for checking the overmultiplication of *Bacteria* — a kind of extra-cellular phagocytosis where this plasm plays the role of the cytoplasm in, for instance, a blood leucocyte. Or, in view of the fact that food inception is a deliberate and slow

process in all *Choanoflagellata*, it may also be a simple means of preventing clogging of the collar surface by a surfeit of adherent *Bacteria*.

Many of these agglutinations have been watched for long periods at a stretch, and it was noted that they resisted disintegration for several hours, and that the contained *Bacteria* showed no sign of life at any time. This supports the writer's belief that any *Bacterium* which has become completely enveloped by this plasm is quickly killed, and, if selected as food, undergoes a crude kind of pre-digestion before being passed into the sarcode ; the action of this plasm, supplemented by that of the food-engulfing and food-receiving vacuoles, being akin, albeit remotely so, to that of the salivary secretion in higher animals.

Apart, however, from all these considerations, the writer regards this excessive production of collar-plasm as strengthening his explanation of the apparent presence of up-flowing collar-currents. Since this surplus of plasm is obviously the result of extra stimulation to the production of such currents by the bacterial congestion on the collar ; a condition of things which again has its remote analogue in the so-called animal kingdom, where excessive secretion of mucus under similar conditions prevails. The collar, in fact, here functions as a rudimentary mucous membrane with certain other complex attributes.

TEGUMENTARY STRUCTURES. — The essentiel differences between these cell coverings are still being debated by DE SAEDELEER and the writer. Exhaustive bio-chemical methods are, however, necessary for their complete elucidation.

In certain new and striking marine species, which the writer has discovered and describes later, and in some *Salpingoecidae*, there is considerable difficulty in separating the two types of tegumentary structures at present recognized — the loge or mucous envelope and its various manifestations, and the lorica — owing to the duplication or absence of one or both, and the apparent simulation or substitution of the one by the other.

For instance, in *Diaphanoeca grande* (gen. et spec. nov.) the cell is protected first by a close-fitting tegument, which DE SAEDELEER identifies as, probably, an inner lorica ; this tegument is surrounded by a thick layer of jelly, — the mucous envelope or jelly-loge; which, in turn, is bounded by a large and delicate but rigid, extremely transparent outer shell strengthened by costae, of elegant shape, and having characteristics common to both loge and lorica — a loge-coque or pseudo-lorica, in fact.

Another new marine genus *Acanthoeca*, is, morphologically, even

more striking. Here the lorica is much elaborated (see description and
figure 5) and is of the most resistant type, since it has been observed
to survive maceration in sea-water for 10 days without the slightest
change in any of its parts. The whole structure is therefore probably sili-
ceous — a true lorica.

Lastly, in *Salpingoeca marssoni* and other related species where the
body of the lorica is apparently a double structure, the whole or part of
which is coloured a shade of brown, we find either that the inner tegu-
ment is the true lorica and the outer one a hardened loge : or, as the
writer believes, we have, as the inner tegument, a very delicate pseudo-
lorica terminating anteriorly in an equally delicate chimney-shaped neck,
and a second, very thick and obviously chitinous pseudo-lorica outside.

The general conclusions arrived at by the writer in regard to these
teguments in the *Choanoflagellata* as a whole are as follow.

The main or outermost protective investment may be :

1° A simple layer of colourless jelly-plasm of limited depth, the sole
protective investment in the *Codonosigidae* — *the mucous envelope or
loge.*

2° A transparent, or coloured, sometimes opaque, thin or thick, horny
(chitinous) structure formed either on the lines of the true lorica or cons-
tituting the outer layer of a doubled structure — the *pseudo-lorica or
loge-coque.*

3° A siliceous resistant structure, usually vasiform — *the true lorica.*

Any of these may function as an auxiliary investment. Each is the
result of a plasmic secretion of definite bio-chemical composition produ-
ced by the cell sarcode.

The mucous envelope, owing to its extreme hyalinity, is normally
visible only after the application of stains or other reagents. It appa-
rently invests the cell as an egg-cup does an egg ; i. e., only partially.

The respective resistant and other physical characteristics of chitin and
silica frequently serve to differentiate visually the two rigid investments.
The chitinous, pseudo-lorica readily crumples, assumes a brownish
colour with age, and, unless of considerable thickness, survives only a
short time the death of the cell which produces it.

The siliceous, true lorica never crumples, remains uncoloured, and
retains its rigidity almost indefinitely.

THE PEDUNCLE. — In considering the derivation of this anchoring
and supporting appendage we are confronted, as in the case of the
tegumentary structures, with the fact that, in a collective sense, the
choanoflagellate cell has at its disposal a whole gamut of plasmic

secretions, each of which normally produces some definite structure of definite chemical and physical constitution, or serves some definite function.

In DE SAEDELEER's opinion the true peduncle — the initial formation of which (1), as the present writer has observed in *Codonosiga* and in *Dicraspedella*, is apparently the result of the confluence of several basal pseudopodes emitted and exhibited particularly during the free-swimming stage — is produced by a secretion quite distinct from that of the mucous envelope, pseudo-lorica and lorica; and the present writer agrees with him. The remarkable flexibility and rigidity of the true peduncle point to a secretion having the joint properties of the pseudo-lorica and the lorica, but beyond this suggestion the writer will not now venture. He will, however, direct attention to the complexity and to what he considers to be the evidence of the tubular character of the peduncle.

In *Salpingoeca cardiforma* (var. *tulepoda*) the peduncle supports a pseudo-lorica of elegant form and terminates at the exact centre of a large and conspicuous, dome-shaped foot-swelling. This foot-cushion is of quite distinct plasmic constitution from that of the peduncle, as its dark-brown colour indicates; and, although attached to a fully developed lorica in every case, it exhibits considerable variation in size. It also persits long after the dissolution of its superstructure. From observations on a young cell, following its separation soon after fission, from the parent cell, it was seen that, primarily the peduncle is the result of the fusion of a number of filiform pseudopodes, which are doubtless different constitutionally, but which resemble those emitted at the posterior of the cells of *Codonosiga* and *Dicraspedella* for the same purpose, just before they come to rest after a period of free-swimming.

During the gradual increase in length and slenderness of this peduncle the lorica is seen to develop from the cell's anterior downwards, and it eventually joins up with the peduncle at a point a little below the cell's posterior, thus appearing to cut off the short length of peduncle which lies between the bases of cell and new lorica, and this length of peduncle appears actually to be withdrawn, presumably into the sarcode, thus proving its pseudopodic nature. Something like this has been observed by HOOGENRAAD to occur in *Hedriocystis*, a Heliozoan of *Clathrulina* type (2).

(1) Cf. DE SAEDELEER, Note II, p. 260, foot-note 3.

(2) H. R. HOOGENRAAD, 1927. Beobachtungen über den Bau, die Lebensweise und die Entwicklung von *Hedriocystis pellucida* Hertw. et Less. In *Arch. für Protistenk*, cf. p. 336-337.

How, then, is the rest of the peduncle constituted and the cushion formed ?

Three answers suggest themselves : (1). The peduncle below the completely formed lorica remains pseudopodic for some time, but gradually hardens and becomes tubular by internal shrinkage. The short length of peduncle, which was seen to be withdrawn, is replaced by, or leaves behind, an invisible funnel down which the special cushion-secretion is projected into the tubular peduncle and wells out at the latter's further extremity, the column or film of cushion-secretion, which remains inside the peduncle, adding to the latter's rigidity (2). The pseudopodic peduncle is a temporary one, and the lorica, instead of ceasing its growth when the pseudo-peduncle is reached, continues it down to the substratum, thus producing a hollow peduncular extension up which the pseudopode is withdrawn, and the subsequent events. as described in (1), take place. Or (3) and perhaps the most probable : the cushion-plasm is actually poured out through the main (tubular) pseudopode at the moment the latter attaches itself to its substratum, but, owing to its extreme hyalinity, remains invisible until age, or the presence of iron or sulphur, colours it a varying shade from brown to nearly black. In each case the size of the foot-swelling and the length of the peduncle are governed by the actual amount of secretion poured out, the comparative vigour of the cell's internal ressources being responsible for the final result. And, it may be added, for a similar reason the true peduncle in the illoricate genera shows considerable variations in length in the same species. For instance, in a species of fresh-water *Codonosiga*, seen by the writer in November 1928, the peduncle reached the astonishing length of approximately 140 μ, the cell's length being 12 μ. A specimen of the marine genus *Dicraspedella*, with a cell length of 6 μ had a peduncle 62 μ long. At the same time the writer agrees with De Sae-deleer that, for a given species, the peduncle preserves a fairly constant average length and an even more constant thickness.

The above instances may therefore be abnormal, unless it transpires that this abnormality is a definite characteristic worthy of conferring separate species rank on the respective cells.

With regard to the constitution of peduncular structures other than the true peduncle e. g. those which are apparently simple extensions of the lorica or pseudo-lorica the writer again can only offer a guarded opinion. He permits, himself, however, to generalize thus :

The peduncle which is derived from the true lorica never attains any considerable length and is never the result of the coalescence and sub-

sequent extension of posterior pseudopodes. It is a simple, more or less attenuated prolongation of the lorica, and is probably hollow throughout its length.

The peduncle which is derived from the pseudo-lorica also never exceeds a moderate length, but may result either from posteriorly-developed pseudopodes or from the simple prolongation of the base of the lorica ; or may even be a combination of the two processes. Obviously to determine these derivations one must actually observe, as the writer has done, the development, from beginning to end, of the young cell. Even this may be perplexing and inconclusive without the aid of reagents ; and we have yet to find a reagent or reagents which will with certainty differentiate the whole gamut of secretions which go to produce the slightly or widely differing tegumentary and other structures in the *Choanoflagellata.*

To further illustrate this complexity, in its application to peduncular constitution, the writer may here refer to the evident difference, as exhibited in the arborescent genus *Codonocladium* (*Codosiga umbellatum* of Saville KENT), between the secretions responsible for the main trunk and the extremely rigid branches of the peduncular system, on the one hand, and the short, plastic, and probably pseudopodic pedicel by which each of the cells is attached to the summit of one of the more slender terminal branches, on the other.

The trunk, or main supporting peduncle, of the colony is the product of a single cell, and no doubt originated from the coalescence of several posteriorly-developed pseudopodes, since amoeboïdity, accompanied by the protrusion of pseudopodes, all pointing directly backwards, have been seen by the writer when the cell, owing to irritation, detaches itself from its supporting branch.

The process then is probably as follows :

The original cell secretes a peduncle and divides longitudinally, and each of these cells divides again, four cells only being produced at this stage. The result of the peduncle-secreting activity of these four cells is the first branch system. Each of these branches supports a maximum cf four cells which continue the branch system to the next stage, the one that follows this being the last. So that the final strengh of the colony, if every cell survives, will be 266 cells.

But how are the components of this extremely rigid and probably siliceous supporting structure produced ?

Are the main trunk and its branches each the direct product of a pseudopode ? or is the latter a temporary support around which the sili-

ceous secretion is poured, ultimately forming the rigid branch up the hollow centre of which the pseudopode withdraws, and from the end of which, when completely formed, the cell partially detaches itself — the incompletely withdrawn pseudopode constituting its means of attachment ?

Observation seems to bear out the latter suggestion, and its similarity to one of the suggestions made in connection with the formation of the peduncle and its cushion foot in *Salpingoeca cardiforma* will be apparent.

Reproduction. — Binary subdivision has been observed by the writer in four genera either partially or completely. Gemmation, as described by De Saedeleer and others, has not actually been observed. And sporulation, which Saville Kent professed to have seen, has not been confirmed.

That fission is invariably longitudinal contemporary biologists are agreed, and the writer defers to this consensus of opinion, since it has doubtless been established by protistological inference reinforced by modern cytological evidence. But he would remark that the process in all the loricated species is unnaturally clumsy, and that the production of the hernia, from part of which the young cell is by constriction cut off, strongly ressembles gemmation, just as the same but less obvious process in *Stephanoeca ampulla* (as observed from first to last by the writer), simulates transverse division.

There is usually ample space within the lorica to permit longitudinal subdivision on the straightforward lines followed by the illoricate species. In any case, if the partial emergence of the cell, and the production of the hernia outside the lorica is due, in most of the *Salpingoecidae*, to the narrow neck of the lorica offering a difficulty to the young cell's egress, that difficulty is not present in such a species as *S. inquillata*, where the lorica is very commodious and wide throughout its length ; yet the hernia is duly produced in this species. The daughter cell of *S. ampulla* has a similar difficulty to overcome, namely, the narrowed orifice of the all-enclosing lorica within which subdivision is effected ; but this it does by changing its shape from an ovate sphere to that of a slightly truncated cone.

The reason, then, for this urge for semi-emergence and the production of the hernia (in other words, longitudinal subdivision abnormally and clumsily effected) seems, on the face of it, obscure.

As already stated, the writer has observed part or the whole of the process of fission in several widely differing species.

In the new, double collared species *Dicraspedella* which is closely related to the *Codonosigidae*, longitudinal division is quite normal, and the different stages are clearly displayed and correspond probably in every detail with those seen by DE SAEDELEER in *C. botrytis*.

The cell, when about to divide, gradually lessens in height and at the same time broadens considerably, the collar as well. The flagellum, at an early stage in this widening process, is considerably retracted and a second flagellum is seen to appear and take up the position it will occupy when the cell actually divides; this stage (in view of the intimate connection existing between the flagellum, the nucleus, and the complex region between them) being, of course, associated with the division of the nucleus. The cell and collar then simultaneously divide downwards, the two rejuvenated cells, the products of this division, remaining attached, each by a short, posterior pseudopode, to the top of the common peduncle.

In the loricated species, where the cell partly emerges from the lorica mouth and forms a hernia from which, by constriction and severance, the new cell is produced, it is very difficult to see what happens from minute to minute during the act of subdivision : and DE SAEDELEER's drawings of *Salpingoeca amphoridium* and *S. irregularis* do not, it is feared, help us to visualize precisely what occurs when the original collar and flagellum are divided between the so-called mother and daughter cells.

The writer has seen division in *S. inquillata* several times, and his drawings of this will, he hopes, make clear what his own interpretation of the phenomena is (fig. 26).

He does not believe that the collar is *wholly* retracted and reproduced de novo in this or any other species excepting, perhaps, in the two very distinctive genera *Stephanoeca* and *Diaphanoeca*. During the production of the hernia the collar certainly remains visible all the time. The genesis of the second flagellum and the moving apart of the two flagella have not been observed, and it is probable that the flagellum at this stage is considerably retracted.

At a stage between (A) and (B), that portion of the common collar which lies directly above the cleavage point in the hernia has obviously become extremely thin owing to stretching, and gives way with the deepening of the cleft which cuts off the young cell. This is the stage at which it is most difficult to follow the progress of sub-division, especially from a frontal view. But the subsequent stages (B) and (C), showing the survival of the outer hemi-spheres of the collar in the

two cells and the upward growth of the inner ones, gives the writer's interpretation of what he has seen. And the correctness of this interpretation is apparently confirmed in the subdivision observed and now to be described in the new and remarkable genus *Choanoeca* (fig. 22).

In this genus, represented by but one species at present, the normal, adult cell is entirely without a flagellum until the cell is about to subdivide, when a robust flagellum is produced solely for the use of the young cell ; that is to say, the flagellum is passed on to the young cell simply for locomotory use, and to ensure for the latter a fresh substratum preferably some distance away from that of the parent cell — a provision doubtless connected with the question of adequate food supply.

Fission, in the solitary instance observed, was very clearly presented, and the drawings are almost self-explanatory. The hernia is duly formed, and the collar, enormous in this species, first closes up as at fig. 22 a, then broadens out, and a section of its central portion, on opposite sides, apparently collapses within the cleft of the dividing cell (hernia), since at (D), where the young cell is shown moving away, a short, rather thick, plasmic strand — consisting, the writer believes, of the collapsed sections of the collar — is observed connecting the two cells. This connecting piece, as the new cell moves further away, lengthens and expands, thus becoming more and more attenuated in optical section, and ultimately re-forms the complete collar in both cells.

The flagellum is passed intact, as shown, to the new cell, which, when completely detached, moves slowly away backwards until it encounters some obstacle. Here it remains for a few minutes, the flagellum moving sinuously and languidly. Then, suddenly, the flagellum is thrown into violent movement and the cell swims off, posterior foremost and collar directed backwards, with such speed and such frequent changes of direction and depth as to preclude its further career being followed by the high-power objective needed for noting the above details. No doubt the young cell eventually comes to permanent rest, secretes its lorica, withdraws or throws off its flagellum, and develops its collar to the immense size characteristic of the genus.

Saville Kent evidently did not see the act of fission in *Salpingoeca* (now *Stephanoeca*) *ampulla*, but apparently did see a " flagellate collarless germ " — presumably the product of gemmation in that species — develop into what is possibly the present writer's distinct species *Stephanoeca kenti* (vide Kent's plate III, fig. 21 in his *Infusoria*). If this observation could be confirmed it would be important. In any case it is a remarkable one. But the present writer considers even more remarka-

ble the fact that, in the numerous spreadings he has examined containing *S. ampulla* and *S. kenti*, he has not himself seen any developing form even remotely resembling Saville KENT's figures 19-20.

Fission in *S. ampulla* has been observed by the writer on two occasions, and the following particulars and the drawings combine his results. Reference has already been made to its apparent simulation of simple transverse division, and figures 25, a-f, will bear this out.

The partial emergence of the cell from the narrowed basal portion into the wider, arching, upper portion of the lorica presumes the production of the hernia, although the side-bulging of this was not seen, probably because the position was frontal in each case. Attention is, however, directed to several interesting points : (a) the apparent direct continuity existing between the collars and flagella of the two divided cells : (b) the retaining, by means of a long filament or narrow band, of prolonged connection between the two collars : (c) the change to a suitable shape of the new cell in order to allow of its passage through the constricted mouth of the lorica : (d) the pseudopodic excrescences on the developing lorica seen at the base of the new cell : (e) the development of the lorica from the base of the cell upwards, the progress of which was watched until the complete and perfect replica of the parent was seen to result.

Considering further some of he above points : point (c) bears out the writer's contention that the young cell could make its way out from the neck of any lorica by some such means as this, and thus longitudinal fission could take place entirely within any lorica ; point (e) raises the question : Why does development of the lorica proceed in contrary directions in different genera, since in *S. cardiforma* it has been shown to commence at the shoulder of the cell and proceed downwards ? Lastly, with regard to point (a), the writer has already suggested that, probably in this and its related genus *Diaphanoeca* alone, the flagellum and collar are completely withdrawn and re-developed simultaneously at the point of separation of the two cells. Admitting, then, the real continuity of the two collars and flagella, as shown in fig. 25 c and d, (and the persistence of the long connecting strand (fig. f) referred to in (b) seems conclusive), the writer can conceive of no other process whereby such a result could be attained.

In any case, it is interesting to compare the writer's drawings of *S. inquillata* with those of Saville KENT of this species (plate 6, fig. 1 to 6) and of *S. gracilis* (Plate 6, fig. 28, 29).

It will be noted first that in fig. 29 Saville KENT represents the cell

of *S. gracilis* dividing actually within the lorica, thus supporting the present writer's argument regarding the latter's commodiousness and which therefore should obviate the necessity of the cell's partial emergence from the lorica and the subsequent production of the hernia, but which does *not* — at least in this species and in *S. inquillata*. Next, Saville KENT evidently regarded fission in both these species as being definitely transverse, not longitudinal. Lastly, while unaccountably omitting the flagellum, he represents the actual continuity of the two collars in both species.

On the other hand, DE SAEDELEER in figures 70 and 71 of his " Craspédomonadines : morphologie and physiologie ", shows the young cell in *S. amphoridium* upended above the mother cell, but with its collar and flagellum slightly discontinuous with that of the parent.

For his part, the present writer is convinced that his own observations of the division and development of the collar and flagellum in the species he has described, are as represented.

Which then is correct ? Or have we indeed distinct types of fission ; or, at any rate, different sequelae after fission, in the *Choanoflagellata* ?

SUMMARY OF FOREGOING OBSERVATIONS AND CONCLUSIONS. — FOOD INCEPTION in general is practically identical with that exhibited by certain other *Protomastiginae*, such as *Oïcomonas* and *Amphimonas*, and by the new pantostomatinous species observed by DE SAEDELEER (loc. cit., fig. 94-100), i. e., by the protrusion at the body-surface of sarcodic engulfing-plasm, with or without the production of definite prehensile pseudopodes, either linguiform or as a pseudopodic wave, near the appendage which effects or brings about the capture of the prey, thisappendage being the collar, aided in most species by the flagellum.

FOOD is exclusively bacterial, including, probably, ultra-microscopic forms.

THE COLLAR is believed to be a structure composed of a number of extremely flattened axopodes or filipodes derived from ancestral forms related to the existing heliozoan types represented by *Pteridomonas*, the Cyrtophorines (*Pedinella*, etc.) and the loricated Heliozoan described in the text.

THE COLLAR SURFACE-CURRENTS. — The bi-directional, but strictly axial characters of these are explainable by the assumption that the individuality of the component ancestral filipodes or axopodes has been, to a certain extent, retained, each being the seat of an upward or

downward current produced under the general governance of an intra-sarcodic directive system, but re-acting to local stimuli.

COLLAR-MUCUS. — It is suggested that the rôle of this viscous plasm, apart from its primary function — the capture and retention of prey — is not only bactericidal but may also be remotely akin to that of the salivary secretion.

EXCESS PRODUCTION OF COLLAR-MUCUS is believed to result from the congestion and irritation caused by an accumulation of *Bacteria* upon the collar, and is analogous to that caused by similar conditions on the mucous membrane of the higher animals.

TEGUMENTS. — These are evidently the product of different but definite sarcodic secretions. Three are, with more or less ease, recognizable :

1) *The mucous envelope* or *loge* ; a thin, highly translucent jelly, differing in depth according to the genus or species.

2) *The true lorica* ; the main protective structure in most of the Choanoflagellata, and exhibiting considerable variety and beauty of shape. Shown by DE SAEDELEER to resist the solvent action of all the so-called mineral acids, excepting hydrofluoric, it is assumed to be the product of a secretion with a large siliceous content.

3) *The pseudo-lorica* ; a rigid structure, brown in colour when of considerable thickness, resembling the true lorica in shape but much less resistant. The product of a secretion probably of a chitinous nature, with or without a varying siliceous content. (The writer hesitated before making a distinction between these two lorical structures in view of the difficulty in deciding into which category " border-line " loricas should be placed, and also for the sake of simplicity. It may therefore be deemed preferable to adopt the general name " lorica ", with the definition "the product of a siliceous, silicochitinous, or chitinous secretion ". The differences enumerated are, however, evident in a greater or less degree, and the distinction is therefore admitted tentatively).

THE PEDUNCLE. — A perplexing structure in some cases but, when obviously normal and simple, its derivation falls under two heads : It may be (1) a modified pseudopode, probably the product of a special secretion ; (2) a simple prolongation of the lorica or the pseudo-lorica. The former is a true peduncle, possesses great flexibility and considerable rigidity, and may attain to an extreme length. The latter is merely a peduncle-like extension of the rigid tegumentary structure which it supports, and while, when attenuated, its flexibility is considerable, its

length is quite limited ; in certain species it is probably permanently tubular, in others only temporarily so.

THE SUPERNUMERARY COLLAR. — The writer has searched for representatives of those genera with supposed double-collars which are described and crudely illustrated in LEMMERMANN's, *Flagellatae*, I, (7) and has found no fresh-water forms with a permanent supplementary collar. He has, however, occasionally seen a collar-like structure completely encircling the cell a little below the base of the true collar both in *Codonosiga* and in his new and undoubted double-collared genus *Dicraspedella* (fig. 3) ; but in each case it was found to be merely a temporary protrusion either from the sarcode or of the mucous envelope, induced probably by adverse environmental conditions. The writer, therefore, believes that DE SAEDELEER's criticisms of the three genera *Diplosiga*, *Codonosigopsis*, and *Diplosigopsis* are based on sound reasoning.

In *Dicraspedella* the secondary collar is not a separate appendage with a separate base on the cell, as shown in the above three doubtful genera, but arises at almost a right-angle from the main collar itself (fig. 1).

It appears to perform no particular function, and is therefore regarded by the writer as a useless extension of the hypothetical outer wreath of ancestral axopodes from which it is derived, the distal half of which remains separate from, while the basal half still preserves contact with the main collar. A secondary collar of this type, but of much smaller size, has also been observed in a loricated species (fig. 2) and it is believed that such a rudimentary collar will be found in others.

REPRODUCTION. — Longitudinal fission probably prevails, gemmation is rare, and sporulation has not been confirmed.

The means employed by the loricated cell to effect its longitudinal fission, viz. by its partial emergence at the neck of the lorica with the formation of a hernia from which, by cleavage, the young cell is produced, is clumsy and often simulates, more or less closely, both gemmation and transverse fission.
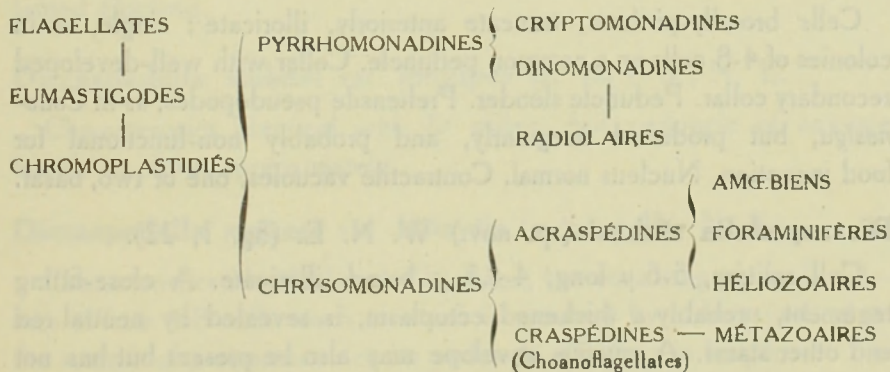
By the observation of binary subdivision in several genera it is concluded that, with the possible exception of those characterized by an all-enclosing lorica viz. *Stephanoeca* and *Diaphanoeca*, the collar and flagellum are never completely retracted ; the collar stretches laterally, gives way down its centre, and this fractured or collapsed section, probably corresponding to exactly half the collar expanse of each of the

divided cells, is reformed by gradual upward development from the base.

The actual growth of the lorica, from beginning to end, has been observed in two widely different marine species *Stephanoeca ampulla* and *Salpingoeca cardiforma*. In the former, development is from base upwards ; in the latter the process is reversed. In each case the growth of the lorica is preceded or initiated by amoeboidity at or near the base of the cell. In *Stephanoeca* the region of the developing lorica is the seat of such amoeboidity, and is a transient disturbance. In the other case the amoeboidity takes the form of definite pseudopodic activity with a definite object, viz., the production of a peduncle.

These two observations are illuminating since they prove : a) a lack of uniformity in direction of growth of the choanoflagellate lorica ; b) the separate constitution of the secretions which respectively produce the lorica and the true, but probably in this exceptional case temporary peduncle.

CONCLUDING REMARKS. — In the purely cytological work yet to be undertaken on the cell in the various choanoflagellate genera further light will no doubt be thrown on all the problems discussed in the foregoing pages. There is, however, bound to be considerable difficulty in this work, since, although the nucleus is relatively large, the cell itself, except in the primitive genera, is small often extremely so; and, apart from the few abundant colonial species, cells are rarely available in the necessary numbers. Nevertheless, in view of the important genealogical position of the group, forming as it does (according to LAMEERE) a direct link between the Flagellatae and the Metazoa (see part of LAMEERE's table below), such investigations are seriously called for and should yield most valuable results.

FLAGELLATES
|
EUMASTIGODES
|
CHROMOPLASTIDIÉS

PYRRHOMONADINES

CHRYSOMONADINES

CRYPTOMONADINES
DINOMONADINES
|
RADIOLAIRES

ACRASPÉDINES

CRASPÉDINES — MÉTAZOAIRES
(Choanoflagellates)

AMŒBIENS
FORAMINIFÈRES
HÉLIOZOAIRES

It is hoped that this paper — the effort of an observer who has throughout preserved an open mind, and thus, perhaps, arrived at certain subversive and startling conclusions — may provide the necessary incentive for a mass attack on the cell as a microcosm, and so bring harmony into the ranks of those biologists who, he believes, are still somewhat undecided as to the identity and function of those perplexing structures and " inclusions " which lie above and within the animal-cell nucleus.

## NEW GENERA AND SPECIES

Apart from the few species discovered by Saville KENT little appears to have been done by subsequent workers in investigating the salt-water Choanoflagellates.

Since 1923 the writer has devoted most of his attention to remedying this neglect, with the gratifying results which usually follow the exploration of an almost virgin biological field.

Saville KENT predicted the probable generic importance of his striking species *Salpingoeca ampulla*, and the present writer has realized this expectation by discovering four other well-defined species of this type. The new genus *Stephanoeca* has therefore been created.

Four other new marine genera have also been discovered and created, each possessing extraordinary and distinctive characteristics ; a new generic separation is suggested for a fresh-water type, and Saville KENT's genus *Lagenoeca* is criticized. A number of forms which are believed to be new species in existing genera have also been discovered, only the more important here being described and illustrated. All have, however, been photographed and provisionally named, and will form the subject of later communications.

**Dicraspedella** (genus novum) W. NEALE ELLIS.

Cells broadly-piriform, truncate anteriorly, illoricate ; single, or in colonies of 4-8 cells on a common peduncle. Collar with well-developed secondary collar. Peduncle slender. Prehensile pseudopodes, as in *Codonosiga*, but produced irregularly, and probably non-functional for food inception. Nucleus normal. Contractile vacuoles, one or two, basal.

**Dicraspedella stokesi** (sp. nov.) W. N. E. (fig. 1, 22).

Cell solitary, 5-6 μ long, 4-4,5 μ broad, illoricate. A close-fitting tegument, probably a thickened ectoplasm, is revealed by neutral red and other stains. A mucous envelope may also be present but has not been demonstrated. Peduncle very slender, 10-30 μ in length. Collar,

with short secondary collar. Sides of main collar almost parallel as in *Colonosiga*. The secondary collar arises at a point roughly one third the distance up the main collar, with which it at first makes almost a rightangle, then turns up at the margin, the resulting structure being a fairly deep trough, with a turned-up edge, encircling the main collar.

The two prehensile pseudopodes are normally produced alternately on opposite sides of the cell at long and irregular intervals. Often they are not visible at all, and not infrequently are produced simultaneously. Moreover, they have not been seen to participate in food inception, and are therefore probably in process of complete suppression in favour of the more direct and deliberate methods exhibited by the higher loricated species.

Food inception in the present species is initiated at the junction of the two collars, the prey being conducted downwards — doubtless by a co-ordinated plasmic current on the opposed collar surfaces — between the outer basal portion of the main collar and the inner basal portion of the secondary collar, direct into the sarcode, thus proving the separate axopodal derivation of the secondary collar, as already suggested.

The action of the flagellum is usually extremely vigorous, causing the cell and upper part of the peduncle to oscillate briskly. For the same reason, the outer collar surface becomes frequently clogged by an accumulation of adherent *Bacteria*. This clogging is countered by the outpouring of an excess of mucus from the main collar in preponderating, upward-flowing currents by which the surplus *Bacteria* are conducted to the collar mouth for ultimate dispersal. This dispersal is slow, since the rejecta have been seen to remain inglutinated in the mucus in the neighbourhood of the collar for considerable periods. This ejected collar mucus is, therefore, not readily soluble in water, and its object is apparently not merely the inhibition but the actual destruction of its contained *Bacteria*.

**Dicraspedella stokesi** var. **longipes** (n. var.) W. N. E.

Characteristics identical with *D. stokesi*, but peduncle of extreme length viz. 60 μ approximately.

**Dicraspedella stokesi** var. **botrytis** (n. var.) W. N. E.

Characteristics identical with *D. stokesi*, but consisting of small colonies of from 4-8 cells, each of which is attached by a short pedicel to the summit of a common, slender peduncle, 20-25 μ in length.

### Choanoeca (genus novum) W. N. E.

Cell solitary, piriform, neck considerably elongated, length of cell (extended) : 4,5 to 7 $\mu$. Lorica vasiform, more or less closely investing cell ; sessile or with short peduncle. Collar comparatively enormous and subject to extreme changes in its angle of expanse. Flagellum entirely absent in normal adult cell, but produced, just before cell-fission, for the locomotory and temporary use of the young cell. Nucleus normal· One contractile vacuole, basal.

Habitat : on Algae in stagnant, salt to brackish water.

### Choanoeca perplexa (sp. nov.) W. N. E. (fig. 8).

General details as above. Diameter of collar at mouth : 12-18 $\mu$ at normal angle of expanse, viz. 75° approx. ; the extreme angles of expanse are 10° and 90° — that is, at right angles with major axis of the cell.

The rapid collapse of the collar from maximum to minimum expanse has been frequently observed, the act strongly resembling the sudden closing of an umbrella, and strengthening the conception of a poly-axopodal structure, and the further conception that the component axopodes, corresponding to the ribs of the umbrella, have retained, to a certain extent, their individuality.

The shape of the lorica is that of a short-necked vase with a slightly everted lip and globular body tapering, more or less abruptly, to a point. The peduncle is obviously an extension, 2-6 $\mu$ long, of the lorica.

Visible food inception is effected by the protrusion of a linguiform pseudopode at any point immediately outside the base of the collar. The *Bacterium* is engulfed on the collar surface, usually at about one-third of the collar height, the collar, during this operation, tilting itself upwards and inwards at the side on which the food is being dealt with. The food is then drawn, by the retraction of the pseudopode, into the sarcode, where it is there seen to be invacuoled, and, thus invacuoled, is propelled, by a wave-like contraction of the sarcode at its rear, to the base of the cell.

It may here be emphasized that this process of food ingestion is basically the same in *Codonosiga*, the significant and important deviation being the purposeful (voluntary) production of the pseudopode in the one (*Choanoeca*) instead of the alternating and semi-automatic (involuntary) production of the two pseudopodes in the other.

It is also here suggested that, in view of the infrequency of observed food inception in this species, ultra-microscopic organisms provide a considerable proportion of the cell's nutriment.

The extreme development of the collar has no doubt led to the entire suppression of the flagellum as a contributory factor in the nourishment of the cell. Whether this is an evolutionary advance or a retrogression is an open question. One fact, however, is clear, viz., the comparative unimportance of the flagellum; a fact which is borne out in the two new Choanoflagellate genera next to be described, and also by what has been observed of its evident pseudopodic character in other flagellates, notably Mastigella. The question of its motility is, however, a different matter, and, in the writer's opinion, cannot be wholly explained by electro-dynamic, electro-chemical, surface-tension (or their combinations), or any other theory in pure physics.

### Stephanoeca (genus novum) W. N. E.

In this and the following new genus the lorica, which completely surrounds not only the cell but its collar and flagellum, is obviously designed to prevent the free access of motile and visible *Bacteria* to the surface of the collar. Moreover, both the collar and flagellum are relatively short, and the latter has never been seen in sufficiently active movement as to produce the requisite currents for bringing food to the collar : in fact, no visible food inception has been observed in either of these genera.

The inference, therefore, is that here again we have evidence that ultra-microscopic organisms constitute the sole food ; or that nourishment, probably in the form of bacterial by-products, is effected by assimilation at the collar surface or surfaces.

GENERIC DESCRIPTION. — Cell, collar, and flagellum completely surrounded by the lorica, which is separated by a waist-like constriction into two communicating chambers, the lower, smaller one containing the cell, the upper, expanded one, the collar and flagellum. In probably all species the whole of the lorica is traversed by either transverse only, or by both transverse and longitudinal costae. Lorica sessile or with very short peduncle. Cell truncate-ovate. Collar and flagellum short, the latter probably functionless, the lorica being obviously designed to shield the collar from free impact of ordinary *Bacteria*. Nucleus normal, conspicuous. Contractile vacuole, one.

### Stephanoeca ampulla (*Salpingoeca ampulla*, Saville KENT) nov. nom. W. N. E. (fig. 12).

The following description of this species by Saville KENT, and his two illustrations (fig, 17, 18, plate III, "Infusoria"), are fairly accurate :

" Lorica sessile, narrow and ovate beneath, expanding superiorly in an inflated, balloon-like manner, the external surface frequently exhibiting even longitudinal sulci or striations ; animalcule, including the hyaline collar, entirely enclosed within the lorica, and attached to the bottom of this structure through the intermedium of a slender, thread-like pedicel ; the smaller posterior portion of the lorica enveloping the body, and the balloon-shaped anterior one the expanded collar of the contained animalcule ".

Saville KENT evidently did not witness the act of fission in this species, and it is possible, therefore, that his fig. 19 is a stage following gemmation. The gradual development of the lorica, after fission, takes place while the young cell is floating away from the parent lorica, and an exact replica of the latter is produced in less than forty minutes (vide fig. 25). Saville KENT also failed to see horizontal costae or striae in this species, and to note the continuation of the longitudinal " striae" down to the base of the lorica. These and other observational failures and inaccuracies — notably those connected with food inception in *Codonosiga* and *Monosiga* — were no doubt due to the comparatively inefficient lenses of his time, since the present writer has had no difficulty in seeing and photographing the " striae " with a Beck 1/10 water-immersion objective with $6 \times$ projection ocular, and revealing them as definite costae.

The revised description of this species therefore is as follows : Cell truncate-ovate, average length 5 μ, width 4.5 μ ; when adult, almost filling lower part of lorica; when immature, and therefore smaller, a short anchoring filipode occasionally connects the bases of cell and lorica. Collar and flagellum short, projecting into the upper expanded part of lorica. Movements of flagellum languid, never of such a character as to produce definite food-bringing currents. Shape of lorica resembles that of a crown, hence the generic name ; sessile. Average total length of lorica : 15 μ, upper portion 9 μ, of lower 6 μ, greatest width of upper portion : 8.5 μ, of lower 5.5 μ. Diameter of mouth of lorica : 4 μ. Number of costae : 16 longitudinal, extending from top to bottom of lorica ; 8 transverse, on lower part of lorica.

Habitat : on Algae in salt or brackish, stagnant water.

## Stephanoeca diplocostata (sp. nov.) W. N. E. (fig. 13).

Resembling *S. ampulla* in shape of cell and lorica, but a somewhat smaller species and differing in the number of costae, eight of which (zonal) are in pairs on the upper part of lorica. Sessile.

Average measurements. — Total length of lorica : 12 $\mu$ ; upper portion : 7 $\mu$ ; lower : 5 $\mu$ ; extreme width of upper division of lorica : 7.5 $\mu$ ; lower :4 $\mu$ ; diameter at lorica mouth : 4 $\mu$.

Longitudinal costae : 13 ; transverse (on upper portion of lorica) : 8, in 4 close pairs, set regularly at intervals of 2 $\mu$, commencing at lorica mouth ; 4 single costae cross the lower part of lorica equidistantly.

Habitat : on Algae in salt stagnant water.

**Stephanoeca kenti** (sp. nov.) W. N. E. (fig. 11-11a).

It is probable that Saville KENT's fig 21 represents this species, but he regarded it as a developing stage in *S. ampulla*, and, as in the case of the mature form of that species, he quite failed to see the transverse costae which, on the lower part of the lorica, are even more conspicuous than in *S. ampulla*. The species is a distinctive one and is often found in association with *S. ampulla*, from which it markedly differs in shape and in the greater robustness of its lorica.

General characteristics typical of the genus, but upper part of lorica more elongated than in the preceding species, the lower part comparatively smaller, and tapering basally into a short peduncle. In addition, only transverse costae are apparently present. These traverse both upper and lower divisions of lorica at short, regular intervals, their numbers being, it is believed, 15 in the upper and 8 in the lower part, the former being extremely delicate.

Measurements. — Total length of lorica : 15-17 $\mu$ ; of upper part : 10-11 $\mu$, lower : 5-6 $\mu$.

Extreme width of lorica (upper) : approx. 8 $\mu$ ; at waist : 5 $\mu$, at mouth : 3.5 $\mu$.

**Stephanoeca constricta** (sp. nov.) W. N. E. (fig. 14).

This species was discovered at the beginning of 1923, and the following details are from the only example so far encountered. Unfortunately it was found before the writer had enlisted the corroborative services of photography. It is, however, a wellmarked species as fig. 14 shows.

Conforming broadly to the generic description, it is distinguished as to the lorica by the pronounced waist, the globular form of the lower chamber and the incurving of the upper chamber. Costae not noted. A delicate peduncle, apparently continuous with an anchoring pseudopode within the lorica, is present.

Total length of lorica : 12 $\mu$ ; upper portion : 7 $\mu$, lower : 5 $\mu$ the respective extreme widths having the same measurements.

Diameter of lorica mouth : 4 μ. Length of cell : 4 μ.
Habitat : on Algae in stagnant sea-water.

## Diaphanoeca (genus novum) W. N. E.

This genus has been created in order to accomodate and segregate
species with loricas of the remarkab'e form and delicate structure presen-
ted by the first species, discovered by the writer in December 1922.

As fig. 10 shows, the lorica is of the all-enclosing " ampulla " type,
but differs from the latter in several important particulars, notably in the
reversed position of the expanded portion, and in the fact that the cell,
with its collar, is wholly contained within this expanded portion, in the
centre of which it appears to be suspended without visible means of
support — at any rate in the case of the larger of the two species at
present known. This larger species also shows a reversal in the relative
importance of the longitudinal costae, and offers distinct evidence that
most of the transverse costae are in course of elimination. In fact, so
delicate are all the costae (excepting, perhaps, the " waist-line "
costa) in this species that both kinds may be undergoing suppression, as
appears to have actually happened in the smaller species, where no
costae have as yet been demonstrated.

Generic specification. — C∍ll broad, truncate-ovate. Lorica flask-
shape, the lower bulbous portion surrounding and containing both cell
and collar, the upper portion extended into a thick neck which bulges
slightly medianly, then turns inwards at the lorica mouth ; sessile, and
readily detached from substratum. Collar and flagellum short. Nucleus
normal. Contractile vacuole : one, or two.

## Diaphanoeca grandis (sp. nov.) W. N. E. (fig. 10).

General characteristics as generic description. Cell closely invested
by a firm ectodermal layer or, possibly, a primary lorica. Collar short,
with very broad base-line which, in some individuals, seems to lie a
little below the margin of the flattened anterior of the cell. This and the
central position of the cell within the lower, expanded part of the lorica
is very characteristic.

Longitudinal costae 13, radiating equi-distantly from the exact polar
centre of the base up to the lorica mouth. One thick, zonal costa encir-
cles the lorica at a point where the neck-like differentiation of the lorica
ends, while three much finer costae encircle the lorica, in a somewhat
zig-zag fashion, near its base. The distal margin of the collar appears
to terminate at, and actually make contact with, the above median

costa ; in fact the writer almost convinced himself that there was actual adhesion between it and the collar rim and that by this means the cell retained its central position. But, as De Saedeleer points out, it is perhaps more probable that the cell is imbedded in a jelly-like secretion (i. e. an exaggerated mucous envelope), which fills up the lorica around and below it.

It must, however, be said that, although the hyalinity of the lorica in this species is extreme, no definite evidence of the existence of this jelly-layer has so far been obtained, beyond the fact that the cell *is* suspended by some means other than the usual intra-lorical peduncle or pseudopodes, neither of which has been detected.

Mention may also be made of a curious halo-like, annular structure which has been often observed, usually at the collar-mouth, but frequently a little distance above or below it, in this species and also in *Stephanoeca*. This ring is of common occurrence, and once, in *S. ampulla*, it took the form of a short cylinder, apparently made up of four rings, suspended horizontally above the distorted and moribund cell, the collar and flagellum of which had disappeared.

It is significant that this horizontal and detached ring-like structure is confined to the two genera in which the cell and its appendages are completely surrounded by the lorica. But what of its constitution and origin ? There is obviously some sort of connection between it and the collar and it is probably a special manifestation of outpoured surplus collar-plasm produced as suggested later (vide fig. 10a, 11a, 12a).

The flagellum is thick and short and invariably languid in its movements.

Inception of visible food has not been observed. The situation of the collar renders normal food inception practically impossible, and it is concluded that the usual inter-action of flagellum and collar to this end is wanting in this as in the preceding genus of similar type.

It is also possible that the inner surface of the collar may, after all, play a large part in effecting the cell's nutrition in both these remarkable genera in the way suggested in the prefatory remarks on the genus *Stephanoeca*.

If the inner collar surface has indeed taken over the normal duties of the outer one, the presence of the detached ring structure (referred to above) *within* the confines of the collar is explainable. The writer conceives the surplus plasm composing this ring to have been thrown off as a film which, though usually issuing from the collar-mouth, may do so from any zone on the inner collar surface and, as it does so, turns over

on itself and becomes detached when the (ultra-microscopic ?) bacterial congestion is relieved ; the whole process being reminiscent of the formation of a smoke-ring. In fact it now occurs to the writer as probable that, in every case where excess of collar-mucus is poured out, this ring structure would be produced. The violent lashing of the flagellum, however, prevents its formation in most species, just as the languid, non-purposive movements of the flagellum permit it in these species with all-enclosing loricas.

Average measurements. — Length of cell : 8 μ ; width : 6 μ ; height of collar : 6 μ ; length of lorica : 30 μ (extremes observed : 24 μ and 38 μ) ; extreme width of lorica body : 20 μ ; extreme width at bulge in lorica neck : 9 μ ; diameter at lorica mouth : 7 μ ; longitudinal costae 13 in number, transverse costae : 4. Nucleus normal. Contractile vacuole : one or two.

Habitat : stagnant brackish water. Feeble means of attachment to Algae among which it is frequently found lying loose.

### Diaphanoeca parva (sp. nov.) W. N. E. (fig. 9).

This species was discovered as recently as Oct. of this year (1929) and one individual only has so far been found. This, however, was successfully photographed and the following data obtained. Lorica flask-shaped, delicate ; costae apparently absent. Cell and collar confined to the lower bulbous portion of lorica as in preceding species ; the cell, however, is not suspended centrally but occupies a position at the extreme base of the lorica. The collar is somewhat short and terminates distally at apparently the same height up the lorica as the waist-line costa in the larger species, but in the present species no such costa could be detected.

The flagellum is relatively longer than in D. grande and its movements are more energetic, although not of a character to produce food-bringing currents.

This individual was kept under observation for 72 hours and no change in the lorica occurred and little change in the cell itself, thus proving it to be a distinct species and not an immature stage of D. grandis, as was at first suspected. Measurements, length of cell : 3 μ ; width : 2,5 μ.

Length of lorica : 10 μ ; extreme width : 5.5 μ. Owing to the extremely small size of cell and the presence of debris around the base of lorica neither nucleus nor contractile vacuoles could be distinguished.

## Acanthoeca (genus novum) W. N. E.

This is probably the most distinctive of the new genera now being described. The small species was discovered in June 1928 on Algae taken from brackish marsh-water near Appledore, Devon. The large species was first encountered in Dec. 1924 on marine Algae sent to the writer from the Plymouth Biological Station, and, although several photographs were obtained at the time of the cell, extended and retracted, and of the two outermost spines, and the presence of other spines was suspected, the true structure of the lorica was not revealed until 4 years later, when numbers of empty loricae were found on further ma'erial sent from Plymouth and still later from the Port Erin Biological Station.

Cell, globular, almost completely filling lorica and normally extended anteriorly into a long neck. Lorica of salpingoecidous type, but characterized by an extraordinary development in the form of a circle of spines or spine-like setae, radiating more or less vertically from the outer margin of the flattened shoulder of the lorica. Sessile or pedunculated.

## Acanthoeca brevipoda (sp. nov.) W. N. E. (fig. 6-7).

General characteristics as generic description. Lorica broad, the body bowl-shaped ; the neck salpingous with slightly everted lip and median bulge ; on marine *Oscillatoriae* and other filamentous Algae ; very short peduncle that may be sometimes absent.

Average measurements. — Height of broad part of lorica : 4.2 μ.
Extreme width broad part of lorica : 4 μ.
Length of neck of lorica : 2 μ.
Number of spines : 9-13 μ.
Length of cell (neck extended) : 6 μ.
Extreme width of cell : 3.5 μ.
Nucleus normal. No contractile vacuoles seen.

## Acanthoeca spectabilis (sp. nov.) W. N. E. (fig. 5).

As preceding species, but lorica on more robust lines and more elongated, tapering at the base to the sturdy peduncle. A chitinous foot-swelling of enormous size is sometimes present at the base of the peduncle. Cell-body globular, almost filling the lorica and extended anteriorly into a neck sufficiently long to allow of the collar being expanded well above the trumpet-shaped neck of lorica ; a basal anchoring pseudopode has been observed.

Nucleus conspicuous and normal. Contractile vacuole : one.

Average measurements. — From base of lorica to bases of spines :
10 μ.

Length of lorica neck : 4 μ.

Extreme width of lorica : 6 μ.

Length of outer spine : 10 μ.

Number of spines : 12.

Length of peduncle : 12-15 μ.

Extreme width of peduncle base (when present) : 14 μ.

Average depth of peduncle base (when present) : 3 μ.

Length of cell (extended) : 10 μ.

Extreme width of cell : 5.5 μ.

The writer may here say that he considers it remotely possible that Saville Kent's *Salpingoeca campanula* and Prof. James-Clark's *Codonoeca costata* to which he refers may be the respective interpretations of these two workers of this present species. But as Saville Kent's species has apparently not been met with since its discovery, and no data are available to the writer of Clarke's species, this bald record of an impression must suffice for the moment.

Suggested generic separation of certain salpingoecidous types. — The writer has long considered that a number of species, of two distinct types, now included in the genus *Salpingoeca* should be removed from that genus and placed under separate generic headings.

The names he now suggests for these two genera are *Diploeca* and *Pachysoeca*.

## Diploeca (genus novum) W. N. E.

Species characterized by the extreme delicacy and destructibility of the lorica neck with an accompanying brownish coloration. This genus will therefore include *S. marssoni, angulosa, placita* (sp. nov.) and probably *minor*.

The writer believes that all these, and possibly others, possess an inner lorica strengthened, apart from the neck, by a much thickened outer lorica ; and he bases his belief on his observations on a species he first encountered in Feb. 1922 (which he concluded was *S. marssoni* but which he now considers to have been *S. angulosa*) and his new species *Diploeca placita*.

When viewed in lateral optical section the greater part of the lorica wall of each of these, when sufficiently transparent, is seen to be of considerable thickness ; and that part of this thickened wall which appears

to embrace the base of the delicate neck is distinctly everted, forming a lip-like rim.

DE SAEDELEER does not show (1) this everted rim in his *S. angulosa*, but it is quite a prominent feature, and has been repeatedly confirmed in the writer's similar species.

The writer conceives a lorica of this partly-thickened type to consist of two loricas ; an inner, extremely thin one, of which the chimney-shaped neck is the anterior continuation, and an outer, comparatively very thick one, with a rudimentary neck in the form of the everted mouth.

This double structure is obviously the product of two different secretions, and, it may be assumed, the thick lorica is secreted first, followed by the other in the shape of an intimate lining to the former, with the production, at the same time, of its extension — the salpingous neck. Or, having regard to the cell's secreting powers, the two secretions may be produced on more straight forward and expeditious lines, commencing with the formation of the neck and followed by the simultaneous production of the rest of the lorica.

With regard to the external jelly-covering of the lorica, stated to be present in *S. marssoni*, the writer can only say that there is no indication of such a covering in the two species under discussion ; and, in view of this doubly protective lorical investment, it would seem to be superfluous.

Characteristics of this new genus. — Cell small, broad or oval, with narrow, long, and flexible neck. Collar normal in size, but with large angle of expanse. Lorica sessile, consisting of two in close contact; the outer, thick, more or less everted at its extreme anterior, and closely enveloping the inner lorica up to the base of the trumpet-shaped neck, this neck being the actual continuation of the inner lorica. Each lorica is probably the product of a chitinous secretion, since the inner one, as represented by its neck, is too readily destroyed to be siliceous, while the outer one, although considerably resistant owing to its thickness, is usually of a varying shade of brown —— a coloration indicative of chitin. The double lorica is therefore what has been termed a pseudolorica.

## Diploeca placita (sp. nov.) W. N. E. (fig. 21).

This species was discovered in a gathering from the Victoria Regia

(1) Cf. DE SAEDELEER, I, p. 145 : " Disons enfin, que le col hyalin était généralement situé sur un épaississement de la région apicale du ventre de la coque, ce qui se voit bien en vue polaire... ".

tank at Kew gardens in 1924 and resembles Saville Kent's *S. amphora*, but is smaller, with distinct double-lorica which is rounded, tapering at the base. The writer has a photograph showing nearly all the generic features, particulary the extreme contrast between the very delicate and hyaline lorica neck, and the even and great thickening of the lorica wall beneath it.

Cell, oval, with long neck extending well above the lorica mouth ; partly or wholly filling lorica.

Lorica proper, elongate ovate, brown in colour, and sessile. Nucleus normal. One contractile vacuole.

Length of thickened (double) lorica : 8 $\mu$.

Length of neck of lorica : 2 $\mu$.

Greatest width of lorica : 4 $\mu$.

Cell extended : 8-9 $\mu$.

Cell width : 3 $\mu$.

Habitat : fresh, stagnant water, on Algae etc.

## Pachysoeca (genus novum) W. N. E.

Under this generic name it is proposed to include all species with an obviously thickened lorica, either brownish or colourless, but which, in lateral section, do not exhibit an abrupt lorical thickening (with an everted edge) at the root of a delicate, extremely hyaline, lorical neck.

Such species as *S. massarti*, *S. convallaria*, and the writer's two new species, now to be described, are typical.

## Pachysoeca longicollis (sp. nov.) W. N. E. (fig. 20).

A colony of three individuals provided the following details, but debris obscured their basal regions rendering impossible the proper scrutiny of the cell.

The cell appeared to completely fill the lorica, up the abnormally long neck of which its anterior end is extended a little beyond the lorica mouth.

The lorica neck and its everted rim are of robust build, and the lorica body-wall appears considerably thickened, the lorica itself being sessile, with a broad, flat, or curved base.

Average measurements. — Height of lorica body : 4 $\mu$.

Length of lorica neck : 6 $\mu$.

Extreme width of lorica : 5.5 $\mu$. Collar and flagellum normal.

Habitat : fresh water, on Algae, associated with *Myriophyllum*.

## Pachysoeca depressa (sp. nov.) W. N. E. (fig. 18).

Lorica closely sessile with broad, slightly concave base, the wall of lorica-body thickened but quite colourless. Neck of lorica has a slight median bulge and appears to arise from a concave diaphragm which forms the top or shoulder of the lorica body. The edge of this concavity is seen, when viewed laterally, as a slightly curved line crossing the lorica.

The cell usually completely fills the lorica, including the neck, when extended. Collar, flagellum, and nucleus normal.

Height of lorica (including neck) : 7.5 $\mu$.

Length of lorica neck : 4 $\mu$.

Extreme width of lorica : 7 $\mu$.

Habitat : on Algae in salt marsh-water.

# NEW SALPINGOECIDOUS SPECIES

## Salpingoeca megacheila (sp. nov.) W. N. E. (fig. 16).

This salt-water species was first seen in Jan. 1924 and has been encountered at rare intervals since. The lorica is characterized not only by the enormous development of its mouth, but by what appears to be a short ridge-like projection within its throat (see fig. 6). The photograph shows also two striae or costae crossing the lorica neck at almost the same position as this projection. The latter may, therefore, merely be an optical effect produced by these costae. In any case there is some unusual structural feature here which requires elucidation.

Cell globular, sometimes completely filling lower part of lorica; when smaller, an anchoring filipode connects the bases of cell and lorica.

Lorica globular, the neck of which bulges slightly at the middle and then widens out into the very large mouth.

Peduncle sturdy, frequently tilted obliquely, the lorica, however, remaining vertical.

Collar, flagellum, and nucleus normal. Contractile vacuole : one.

Average measurements. — Total height of lorica : 12 $\mu$.

Height of lorica body : 6 $\mu$.

Greatest width of lorica body : 6 $\mu$.

Height of lorica neck : 6 $\mu$.

Diameter at lorica mouth : 8-9 $\mu$.

Height of collar : 8 $\mu$.

Length of peduncle : 5-6 $\mu$.

Habitat : on Algae in stagnant salt-water.

### Salpingoeca cardiforma (sp. nov.) W. N. E.

Because of the extreme development of a cushion-like formation at the base of the peduncle in a variety obtained in March 1929 from Plymouth it was decided to separate this species as the new genus Tulepoda. But the later discovery, in a Port Erin gathering, of an almost identical form, in which such a foot-swelling is either absent or inconspicuous, has led to both being included in the genus *Salpingoeca* provisionally.

Cell globular when not completely filling the heart-shaped lorica. Neck of lorica short, with slight median bulge. Peduncle of varying length, with or without a small pedal swelling. Collar, flagellum, and nucleus normal. Contractile vacuoles : one or two.

Average measurements. — Total length of lorica : 9 μ.
Extreme width of lorica : 6.5 μ.
Length of lorica neck : 3.5 μ.
Length of peduncle : 8-16 μ.

### Salpingoeca cardiforma var. tulepoda (nov. var.) W. N. E. (fig. 17).

Details as for preceding species, but peduncle reaching a greater length, viz 26 μ, and terminating in a smooth, brown, domed swelling varying in diameter between 4-8 μ and in extreme depth 1-3 μ.

This combination of graceful lorica with slender peduncle arising from the exact centre of the conspicuously large, brown, basal foot-cushion is very striking. The development of the lorica and peduncle in this species has been described in the text. Two alternative suggestions were also offered in explanation of the probable manner of formation of this extraordinary foot-swelling. These questions, however, as well as many others await further investigation.

The lorica in this and the preceding species is often seen to be indented at the side and base, and is therefore of the readily destructible type, the product of a chitinous secretion.

### Salpingoeca de-saedeleeri (sp. nov.) W. N. E. (fig. 19).

(This species is dedicated to Professor Dr Henri DE SAEDELEER to mark the author's appreciation of his up-to-date and enthusiastic work on the Craspédomonadines, and of his stimulating and helpful suggestions to the writer in the course of a voluminous correspondence).

This brackish-water species was discovered in Oct. 1924 and was photographed then, and again in July 1928.

The lorica has two peculiarities, (1) a curiously squat appearance due to its comparatively great breadth, (2) the curves of the body portion appear distinctly angular or facetted.

Cell globular to oval, partly filling the lorica. Lorica neck short, with somewhat large everted lip. Peduncle short. Collar and flagellum long. Nucleus normal. Contractile vacuole : not observed.

Height of lorica body : 6 μ.

Length of lorica neck : 2.5 μ.

Extreme width of lorica : 8 μ.

Diameter at lorica mouth : 4 μ.

Length of cell (contracted) : 5 μ.

Width of cell : 4 μ.

Habitat : on Algae in stagnant brackish-water.

## Salpingoeca huxleyi (sp. nov.) W. N. E. (fig. 15).

This is a well-distributed and fairly common salt-water species.

Cell globular. Body of lorica egg-shaped, with a wide, sturdy neck which bulges towards its own base. This neck appears to be marked off, at its actual base, from the lorica proper by a costa-like ring. This ring is probably an optical effect due to the lorica neck being set in a shallow depression or in-fold of the lorica body.

A further peculiarity is that the neck of the cell is never extended beyond the distal limit of the lorica neck, so that the base of the collar is always within its everted rim.

Collar and flagellum normal. Nucleus conspicuous. Contractile vacuoles : two. Peduncle length variable.

Average measurements. — Length of body of lorica : 8 μ.

Length of neck of lorica : 4 μ.

Width at centre of neck of lorica : 3 μ.

Greatest width of lorica : 6.5 μ.

Peduncle length : 16-32 μ.

## Salpingoeca undulata (sp. nov.) W. N. E. (fig. 4).

This distinctive brackish-water species was discovered and photographed in July 1926 and has not since been met with. The shape of the lorica suggests a transitional stage between those Salpingoecidae having a median bulge in a pronounced lorica neck and those in which the

the lorica in a " free-lance " *S. gracilis.* While in the case of *L. cuspidata,* the " spines " may be peculiar to the " free-lance " *S. amphoridium* ; the shape of the anterior end of the lorica certainly suggests that it is in course of extension into an amphoridium-type neck. On the other hand KENT specifies an " amber-coloured lorica " for his *L. cuspidata,* which points to his *S. amphoridium* being, on this occasion, STOKES' *S. brunnea,* a closely allied species with a brownish lorica which KENT had not differentiated. Or, in view of the abundance of the species *S. angulosa* (also unrecorded by KENT) in which also the lorica is brown and which is often found in company with the other species mentioned and, in view of the apparent duplex character of the lorica in this species, *L. cuspidata* may be a "free-lance" *S. angulosa.* The "spines" of the former would then throw a different light on the development of the outer lorica of the latter, since the " spines " may, after all, have been pseudopodic when KENT saw them, and, later on, might have been seen to coalesce (like the posterior pseudopodes of *Codonosiga botrytis*) and eventually cover the body of the primary lorica.

So much for the writer's speculations in support of his case. If his arguments against the admissibility of the genus *Lagenocca* are proved unwarranted, the opportunity has been offered him to put forward several, to him, interesting facts and suggestions, which may also be of general interest.

(Since writing the above, and when looking back in my notes and sketches for my drawings for the plates, I found that on Jan. 22$^n$ 1925 I observed the free-swimming stage of *Acanthoeca spectabilis.* I have therefore included it at fig. 5a. This would seem to strengthen my case materially).

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## DESCRIPTION OF PLATES

(The total height of lorica is given after each species)

1. — *Dicraspedella stokesi* (gen. et spec. nov.) W. Neale ELLIS, showing, in optical section, one of the two food-engulfing, pseudopodic waves at the height of its upheaval. (Length of cell 6 μ).

1a. — The above species free-swimming and exhibiting posterior pseudopodes.

2. — *Salpingoeca species* (resembling *S. inquillata*) showing short supplementary collar arising from the main collar.

3. — *Dicraspedella* with temporary, collar-like expansion encircling anterior of the cell.

4. — *Salpingoeca undulata* sp. nov. W. N. E. (12 μ).

5. — *Acanthoeca spectabilis* gen. et spec. nov. W. N. E. (14 μ).

5a. — *Acanthoeca spectabilis*, free-swimming.

6. — *Acanthoeca brevipoda* sp. nov. W. N. E. (5.5 μ).

7. — *Acanthoeca brevipoda* (sessile form) (6.5 μ).

8. — *Choanoeca perplexa* gen. et spec. nov. W. N. E. (length of cell 6 μ).

8a. — *Choanoeca perplexa*, with collar flattened out.

9. — *Diaphanoeca parva* gen. et spec. nov. W. N. E. (10 μ).

10. — *Diaphanoeca grandis* sp. nov. W. N. E. (35 μ).

10a. — *Diaphanoeca grandis* with ring, probably composed of a film of surplus collar-mucus, suspended within the collar.

11. — *Stephanoeca kenti* gen. et spec. nov. W. N. E. (16 μ).

11a. — *Stephanoeca kenti* with ring, of surplus collar-plasm at collar mouth.

12. — *Stephanoeca ampulla* Saville KENT. W. N. E. (15 μ).

12a. — *Stephanoeca ampulla* with multiple ring of surplus collar-mucus suspended above cell, the collar and flagellum of which have disappeared.

13. — *Stephanoeca diplocostata* spec. nov. W. N. E. (12 µ).

14. — *Stephanoeca constricta* spec. nov. W. N. E. (12 µ).

15. — *Salpingoeca huxleyi* sp. nov. W. N. E. (12 µ).

16. — *Salpingoeca megacheila* sp. nov. W. N. E. (12 µ).

17. — *Salpingoeca cardiforma* var. *tulepoda* var. nov. W. N. E. (9 µ), the typical species being described in text.

18. — *Pachysoeca depressa* sp. nov. W. N. E. (7.5 µ).

19. — *Salpingoeca de-saedeleeri* sp. nov. W. N. E. (8.5 µ).

20. — *Pachysoeca longicollis* gen. et spec. nov. W. N. E. (10 µ).

21. — *Diploeca placita* gen. et spec. nov. W. N. E. (10 µ).

22. — *Choanoeca perplexa*. Normally without flagellum, one is produced, just before subdivision, for the temporary use of the young cell during its free-swimming stage. A-G., phases in subdivision.

23. — *Dicraspedella stokesi*. Stages in fission.

24. — *Salpingoeca cardiforma* var. *tulepoda*, with recently separated young cell exhibiting posterior pseudopodia.

24a-e. — *Salpingoeca cardiforma* var. *tulepoda*. Development of lorica and showing the replacement of the pseudopodic peduncle by one derived from the lorica.

25. — *Stephanoeca ampulla*. Stages in fission and development of lorica. Note change in shape of young-cell (B and C) to allow of latter's easier egress from lorica mouth. Note also pseudopodic prominences at the base of developing lorica (C. D. E.) and the long connecting strand between collar of young and parent cell (F).

26. — *Salpingoeca inquillata*. Principal stages in fission.

27. — *Salpingoeca inquillata*, with surplus collar-mucus containing inglutinated *Bacteria*, collected at mouth of collar.

28. — *Pteridomonas species* The wreath of filipodes is probably homologous with the choanoflagellate collar.

29. — *Cyrtophora pedicellata* (after Pascher, 1917, fig. 10₂). The axopodes and lobopodes are probably homologous respectively with the collar and the prehensile pseudopodes of *Codonosiga botrytis* (De Saedeleer).

30. — Species of loricated, non-flagellated, tentaculiferous Heliozoon with striking characteristics common to the *Choanoflagellata* (see text under " collar ").

31. — Bi-flagellate monad illustrating food inception by an engulfing pseudopode as is general is the *Choanoflagellata*.