BULLETIN

OF THE

ILLINOIS STATE LABORATORY

OF

NATURAL HISTORY,

URBANA, ILLINOIS.

VOLUME V.

ARTICLE V.—PLANKTON STUDIES. II. ON PLEODORINA ILLINOISENSIS, A NEW SPECIES FROM THE PLANKTON OF THE ILLINOIS RIVER.

BY C. A. KOFOID, PH. D.

Illinois State Laboratory of Natural History,
URBANA, ILLINOIS.
September, 1898.

STATE LABORATORY OF NATURAL HISTORY.

LABORATORY STAFF.

Professor STEPHEN ALFRED FORBES, PH. D.,
Director of State Laboratory and State Entomologist.

CHARLES ARTHUR HART,
Systematic Entomologist and Curator of Collections.

FRANK SMITH, A. M.,
Assistant Zoölogist.

CHARLES ATWOOD KOFOID, PH. D.,
Zoölogist, and Superintendent of Biological Station.

ERNEST BROWNING FORBES, B. S.,
Entomological Assistant.

WALLACE CRAIG, B. S.,
Zoölogical Assistant.

MARY JANE SNYDER,
Secretary.

HENRY CLINTON FORBES,
Business Agent and Librarian.

LYDIA MOORE HART,
Artist.

ERRATA.

Page 136, line 2, and page 182, line 17 from bottom, for '*95a* read '*95*.

Page 226, line 2, page 263, line 17 from bottom, and page 267, lines 2 and 15, for '*98*, read '*96*.

Page 233, line 15 from bottom, for '*82* read '*82a*.

Page 355, line 2 from bottom, for C. *F*. Hudson read C. *T*. Hudson.

Page 389, foot-note, for Vol. *V*. read Vol. *IV*.

Page 457, line 5, for *Genera* read *Genus*.

ARTICLE V.—*Plankton Studies. II. On Pleodorina illinoisensis, a New Species from the Plankton of the Illinois River.* By C. A. KOFOID.

The genus *Pleodorina* was discovered in 1893 by Shaw ('94) at Palo Alto, California, and in May of the following year the species *Pleodorina californica*, upon which the genus was founded, was detected by Mottier ('94) in water from a shallow stagnant pool near Bloomington, Indiana. During the same summer the form also occurred in the Illinois River and its adjacent waters (Clinton, '94), and it has been found in the plankton of these situations in succeeding years from June to September. The distribution of the species in this continent is thus quite extended, and it is not at all improbable that continued investigation of fresh-water plankton will demonstrate that this genus has a cosmopolitan distribution similar to that of some other genera of the family *Volvocineæ* to which it belongs.

On June 16, 1898, a form which may be referred to the genus *Pleodorina* was found in the Illinois River in water entering the stream in large part from Cook's Slough and Quiver Lake. Owing to high water (ten feet above low-water mark) prevailing at the time, a considerable portion of the habitat of the form in question consisted of submerged territory, with shallow warm water abounding in growing aquatic and semi-aquatic vegetation.

This *Pleodorina* could not be found in Quiver Lake collections made on the 7th of June, but on the 16th it was present in the river in small numbers, increasing until the 20th, when a maximum was reached. From this time the numbers decreased until the 27th, when, following a rise in the river, the species seemingly disappeared entirely from the plankton. It was also found sparingly in Thompson's Lake during this period, a large area of slightly submerged territory being at this time tributary to the lake.

Associated with this species in great abundance was *Eudorina elegans*, in all stages of asexual reproduction, and *Pandorina morum* was also present in smaller numbers and in like condition. *Volvox*, *Euglena*, *Phacus*, *Lepocinclis*, *Trachelomonas*, *Dinobryon*, *Synura*, *Mallomonas*, *Uroglena*, *Melosira*, and *Fragillaria* occurred in varying frequency, but only a single specimen of *Pleodorina californica* was found in collections containing the species described in this paper. The animal plankton was represented in the main by rotifers, *Polyarthra* being most abundant, while *Synchæta*, *Euchlanis*, *Pterodina*, *Brachionus*, and *Anuræa* were also present. *Difflugia*, *Codonella*, *Bosmina*, *Cyclops* and nauplii complete the list of the more common associates of this *Pleodorina* in the plankton.

Pleodorina illinoisensis n. sp.

The species here described consists of an ellipsoidal cœnobium or colony of 32, rarely 16 and still more rarely 64, biflagellate cells. The shape is quite constant, occurring in the youngest colony and continuing throughout the asexual cycle until the daughter colonies abandon the gelatinous matrix of the maternal organism. Among the large number examined only a few specimens were seen which approached a spherical form. Measurements of twelve seemingly full-grown colonies from material freshly killed in 2% formalin showed a range of 101 to 137 μ in long diameter, and an average of 113 μ. The transverse diameter ranged from 84 to 102 μ, and averaged 94 μ. Individuals in which the gonidia have begun to divide show a considerable swelling of the hyaline gelatinous envelope. One specimen containing 2- and 4-cell stages measured 178×155 μ, and when the young colonies are ready to escape, the parent may measure as much as 200×175 μ. At the time of escape the young colonies measure 46×38 μ. The measurements of the colonies approach very closely those given by Bütschli ('80–'89, p. 840) for *Eudorina*; viz., 100–150 μ; and the colonies of this genus found in association with the form here described exhibit dimensions almost, if not quite, identical with those above recorded for the *Pleodorina*.

The colony (Pl. XXXVI., Fig. 1) contains, as a rule, 32 cells arranged, as Henfrey ('56) first noted for *Eudorina*, in five circles, two of which are polar and contain four cells each, while eight cells are found in each of the remaining three circles, one of which is equatorial and the other two lie between the latter and the polar circles. The cells resemble those of *Eudorina* in that they are situated in the periphery of the hyaline gelatinous matrix and are not closely crowded together, the degree of separation depending upon the age of the colony and varying considerably in different cases. Their inner ends do not approach the center of the colony as is the case in *Pandorina*. No trace of any protoplasmic connection between the cells of a colony could be detected in the living organisms, nor in material killed in formalin or in chromo-acetic acid and afterwards stained in fuchsin, hæmatoxylin, or Bismark brown. Specimens treated by Zograf's method (1% osmic acid followed by 4% crude pyroligneous acid) or by 1% osmic acid followed by picrocarmine, showed no connection between the cells.

The colony is surrounded by a common gelatinous sheath (*sh.*) increasing in thickness (3.5 to 12 μ) with the age of the organism. This membrane or sheath is of equal thickness in all regions and consists of two parts: an outer, thin, denser, more highly refractive layer (*o.l.*); and an inner homogeneous one (*i.l.*), which shows no traces of the concentric structure found in *Pandorina*. It is within this latter layer that the increase in thickness takes place in the older colonies. It is limited centrally by a thinner and less highly refractive layer (*m.m.*) which encloses the common matrix (*m.*) in which the cells of the colony lie. Frequently among the older organisms there occur upon the posterior end of the colonies blunt, pseudopodia-like protuberances (Pl. XXXVI., Fig. 4) of the sheath, of irregular form and of no constant number. Their position and the fact that they are often, though not always, found in old colonies from which some of the daughter colonies have already apparently escaped, suggest that they may mark the place of exit of the young individuals from the parent. Similar protuberances were observed upon *Eudorina* and *Pandorina*, under similar conditions, in the collections in which the *Pleodorina* under discussion was found. Wills ('80) found that the daughter colonies of *Volvox globator* escaped through a rift in the posterior hemisphere of the parent, and Klein ('89) observed the same phenomenon in *Volvox aureus.* The escape of the daughter colonies in *Pleodorina* has not been observed by me.

The sheath stains deeply in an aqueous solution of methylen blue, more deeply, in fact, than the enclosed matrix, the outer layer taking the deeper stain. It also shrinks to about one fourth its former thickness. This shrinkage, together with that of the central matrix, causes the sheath to wrinkle along lines which bound hexagonal areas from whose centers the cells now project, thus giving the appearance of a division of the surface of the colony into regular polygons. The sheath shows no trace of the layer of radial rod-like structures found by Klebs ('86) in *Pandorina*, but iodine or methylen blue demonstrates a finely granular condition like that described for *Eudorina.* The sheath is traversed by the pairs of flagella which arise from the outer ends of each of the cells.

The matrix (*m.*) is a gelatinous substance of some consistency, filling the colony inside of the inner membrane. In the living colonies, in those which were killed in the various reagents mentioned above and afterwards stained, and in disintegrating material, no traces of any divisions can be detected in this substance that are not due to wrinkling caused by shrinkage. Methylen blue or iodine causes the matrix to show a faintly reticulated or vacuolated appearance due to different densities of staining. That the substance of the matrix has considerable consistency even in the swollen condition found in the maternal colonies, is shown by the fact that the flagella of the young forms, before rotation begins, can be seen to penetrate the matrix of the parent very slowly. Their ends are often blunted or even knob-like and their lateral motion is very limited. The movement of the young colonies through the matrix is a very slow and gradual one, showing the gelatinous consistency of the substance in which they are imprisoned.

The striking feature in the structure of this species, as in the case of *P. californica* (Shaw, '94), is the presence of two distinct types of cells in the colony (Pl. XXXVI., Fig. 1), the vegetative (*v. c.*) and the gonidial (*g. c.*) cells. The presence of these two types of cells at once places this new species in the genus *Pleodorina* rather than in *Eudorina*—which it otherwise closely resembles.

The vegetative cells (*v. c.*) are four in number and constitute the anterior polar circle, being always directed forward in locomotion, as in the other species of the genus. Their number remains the same in the smaller colonies of sixteen cells and in the larger ones of sixty-four. The diameter of these cells ranges from 9.5 to 16.8 μ, twelve cells averaging 12.25 μ. The size of these cells varies even in the matured colonies, measurements at this stage ranging from 9.6 to 15.6 μ. At birth the cells of the young colonies vary in diameter from 3.5 to 5 μ in different parents. In the daughter colonies while still in the maternal matrix, no distinction in size between the vegetative and gonidial cells can be detected, nor can this distinction be made in the younger free-swimming colonies, it being thus impossible at this stage to distinguish the young *P. illinoisensis* from the similar stages of *Eudorina elegans* with which they were associated. When the young colonies have attained dimensions of 46×38 μ, the vegetative cells measure 4 μ and the gonidia 4.8 μ. A like similarity between the two kinds of cells in the young colonies exists, according to Shaw ('94), in *P. californica.*

In structure the vegetative cells (Pl. XXXVI., Fig. 2) are in most particulars similar to the gonidia, described below. They sometimes appear to be a trifle lighter green in color—a difference which may be due to their smaller size. The principal differences lie in the smaller number of pyrenoids and the larger size, both absolute and relative, of the stigma or eye-spot.

As to the fate of the vegetative cells, the evidence at hand is insufficient and conflicting. In three colonies in which the daughters were moving about in the maternal matrix, some having already escaped, the vegetative cells showed very evident signs of degeneration, the contents being shrunken and irregular. In the larger number of instances of this stage under observation the cells appeared normal, showing no trace of degeneration or division. In one instance only have I found a specimen in which the vegetative cells had divided beyond question. This was a colony in which the gonidia had completed their division but had not escaped. Three of the vegetative cells were in the two-cell stage and one was undivided. In two instances matured colonies have been found in which four smaller daughter colonies (of eight and sixteen cells respectively) were present at one pole.

The gonidial cells (Pl. XXXVI., Fig. 3) constitute the remainder of the colony. They usually number 28, rarely 12 or 60, and occupy the parts behind the anterior polar circle of vegetative cells. These cells in most instances can be easily distinguished by their larger size. In form they are spherical, though some specimens in preserved material are slightly flattened on their inner ends. In diameter they range in seemingly full-grown colonies from 15 to 25 μ, averaging in twelve specimens 19.2 μ. Their dimensions just before their division, that is in colonies in which division has begun, also show the extreme range quoted above, the smaller diameter having been found in a sixteen-cell colony. As a rule the gonidia are all of the same size, but occasionally specimens have been found in which one or more dwarf cells occur among them. These are irregular in their distribution and can be distinguished at once from the vegetative cells by their position. Similar dwarf cells were found in both *Pandorina* and *Eudorina*. In matured colonies gonidial cells are frequently found which fail to divide. The gonidia are of a light green color, a trifle darker than the vegetative cells. Their color in general is similar to that in *Eudorina*, and is somewhat lighter than that in *Volvox* and *Pandorina*, with which they are associated.

A distinct cell membrane (*c. m.*) is found about each of the cells. In the living condition and in the material preserved in formalin it forms a highly refractive hyaline layer, about 1 μ in thickness, outside of the green contents of the cell. It stains very faintly in hæmatoxylin and assumes a deep brown tint with long-continued action of iodine and sulphuric acid. In the case of diseased colonies hereafter mentioned the cell membranes persist, often retaining their original form and shape, after the entire disappearance of the contents.

The greater part of the cell contents consists of what seems to be one large chromatophore (Pl. XXXVI., Fig. 2, *chr.*), which occupies all of the cell except the centrally placed nucleus with its enveloping protoplasm, and a slender column (*p. c.*) passing from this region to the anterior end of the cell. In many cells a faintly marked notch or furrow (*fu.*, Fig. 1) is to be detected on one side of the chromatophore at the anterior end of the cell. This seems to mark the line of contact of the sides of the chromatophore which has surrounded the nucleus. In the 2- and 4-cell stages of the gonidial cells the nucleus and the protoplasmic mass are plainly seen to occupy one side of the cell (Pl. XXXVII., Fig. 7, 8), but in the cells of the young colony it again occupies a central position. The chromatophore is uniformly of a bright chlorophyll-green, and shows a finely granular structure under high magnification. In the youngest colonies each cell contains but a single spherical pyrenoid (*pr.*), which occupies a lateral position in the chromatophore, in the inner hemisphere of the cell. In the older colonies the number of pyrenoids increases, as many as twelve having been found. They are scattered irregularly through the chromatophore, and may occur in any part of it. A similar increase of pyrenoids is reported by Shaw ('94) for *P. californica.* In the vegetative cells the number of pyrenoids is often but 2–4, and is, as a rule, less than that of the gonidial cells. In a very few instances as many as eight have been found, and in one old colony the vegetative cells seemed to be packed full of pyrenoids. In the young colonies the pyrenoids have a diameter of about 1 μ, and in the older colonies of 2.5 μ.

The nucleus (*n.*) lies in about the center of the cell in the midst of a mass of protoplasm enclosed by the chromatophore. In mature gonidial cells before division it has a diameter of 7–8 μ, and contains a sub-central nucleolus (*ncl.*) whose diameter is 3 μ. The nucleolus stains deeply with picrocarmine, and is by this means easily distinguished from the pyrenoids, which it resembles in appearance and size. The nuclear membrane is detected with difficulty. It encloses a faintly stained nuclear reticulum (*r.*). In the younger cells the nucleus is much smaller (4–5 μ), the nucleolus is relatively larger, and the reticulum is not evident. In the living cell the nucleolus alone can be seen in the midst of the grayish protoplasmic mass at the center of the cell. The protoplasm is continued from this central region peripherally, in the axis of the cell as a slender column (*p. c.*), to the anterior end, where it includes the stigma and bases of the two flagella. A protoplasmic mantle enclosing the chromatophore was not demonstrated.

The stigma or eye-spot (*s.*) lies at the anterior end of the cell, near its axis, and is often so placed that an equilateral triangle may be drawn with it and the bases of the two flagella as apices. It is of a bright reddish brown color, though in some of the posterior cells the color is often very faint, giving the stigma the appearance of a slightly tinged oil-globule. It is of an elongated hemispherical shape when seen from the side, and has a circular outline when seen from above. Its upper end often projects slightly so as to elevate the cell membrane. The application of killing agents and alcohol soon removes its color, and even in formalin this fades out in the course of a few days, leaving merely a colorless, highly refractive structure. The larger stigmata have a diameter of 2.5 μ and a depth of 2.8 μ, and are to be found in the cells in the anterior part of the colony, especially in the four vegetative cells of the anterior polar circle. Posteriorly the stigmata are less prominent, and are often not to be found at all as brightly colored spots but merely as pale globules whose position alone affords a clue to their real character.

This specialization of the stigmata in the anterior end of the colony occurs also in *Eudorina*, *Pandorina*, and *Volvox*, and Shaw ('94) states that in *P. californica* the stigmata, which are present in the posterior part of the young colonies (in gonidial cells), become less conspicuous and disappear as the colony enlarges and the differentiation of the cells proceeds. This prominence of the stigmata in the anterior end, together with the facts that this end is always directed foremost in locomotion and that the species showing this differentiation are positively phototactic in the vegetative condition when the differentiation is prominent, all point toward the participation of the stigmata in the function of light perception. An interesting phenomenon occurs at the time of the division of the gonidia, for the stigma of the mother cell persists and is passed on through the five successive cell divisions to the outer end of one of the cells of the daughter colony, situated in the margin of the cup which arises from the plate of cells and closes to form the ellipsoidal daughter colony. Inasmuch as this cup always closes from the inside out, that is with the opening directed outward, it is evident that the stigma must traverse the distance between the outer end of the mother cell and its inner end, which corresponds to the outer ends of the cells of the daughter colony. New stigmata arise in the cells of the daughter colony, but being at first very small are thus quickly distinguishable from the persisting stigma. The ultimate fate of this persisting stigma has not been traced.

No contractile vacuole was observed in the living cells, and careful search with a Zeiss $\frac{1}{12}$-inch oil-immersion lens for this structure in preserved and stained material has led to no positive identification of a vacuole. The bleached stigma and what seem to be the enlarged bases of the flagella are the only areas discernible in the anterior end of the cell which at all resemble a contractile vacuole. Shaw ('94) finds in picro-nigrosin material a single vacuole in the anterior end of the young cells of *P. californica.*

The flagella (*f.*) are two in number for each cell, and unite with the cell at the anterior end adjacent to the stigma. The two flagella have the same proportions, and in adult colonies they measure 40 μ in length. In the young colonies they are relatively longer. They are visible on the young colonies shortly after the cup closes, and persist upon the maternal colony during the early divisions of the gonidia.

The locomotion of the colonies of *Pleodorina illinoisensis* is of the type prevailing among other spherical or ellipsoidal genera of the *Volvocineæ*; viz., rotation about the principal or long axis of the colony, either from right over to left or the reverse, frequently with one direction predominating, progression being usually along the line of the axis, the same end of the colony always leading. In *P. illinoisensis* the vegetative pole always leads in locomotion in horizontal, oblique, and vertical movements, and is therefore the anterior pole of the colony. Under normal conditions, when under observation, this species is rarely quiet during the period of growth. While still within the matrix of the mother colony the ceaseless rotation with its frequent reversals begins. Colonies in the life cell, while favorable conditions prevail, can be seen in active movement, jostling one another and their neighbors in their seemingly aimless wanderings. When an object is met which does not yield to their persistent rotation, their movements may slacken for a time to be resumed shortly in some line of less resistance. The rotation of this species is prevailingly from right over to left as the following tables show, which indicate the number and direction of the reversals of rotation in ten individuals in one minute.

Direction	1	2	3	4	5	6	7	8	9	10	Total
Right over to left..	2	1	1	2	1	2	1	5	4	3	22
Left over to right..	2	0	0	1	1	2	1	4	3	2	16

A few days later a second set of observations was made with the following result:

Direction	1	2	3	4	5	6	7	8	9	10	Total
Right over to left..	3+	5±	3±	3±	4+	3+	5+	3+	2+	4+	35
Left over to right..	2−	5±	2±	3±	3−	3−	5−	2−	2−	3−	30

In the majority of instances where the direction of rotation was observed it was from right over to left, the ratios being 22 to 16, and 35 to 30. These tables give some idea of the frequency of change in direction and its variation in different individuals, but do not show the duration of the directions of rotations. This is indicated in a general way in the second table by the plus and minus signs, which show the direction in which the rotation was of longer and shorter duration. In conclusion it may be said that both directions of rotation occur, though that from right over to left is more frequently met with, or, in other words, is of longer duration.

With regard to locomotion in *P. californica*, Shaw ('94) says that "the movement of the plant in the water was followed in the case of a few individuals bearing well-developed gonidia. In swimming through the water the vegetative pole is directed forward and the plant revolves to the right (in observed cases) on the axis connecting the vegetative and reproductive poles. The path is parallel to this axis in upward vertical as well as in horizontal movement." The polarity of this genus thus expressed physiologically in the movements of the colony is accompanied by a corresponding structural differentiation of the cells composing the organism.

In *Gonium*, according to Fresenius ('56), the motion of the colony resembles that of a wheel, progression taking place in the line of the axis of rotation. According to Bütschli ('83–'87, p. 858), locomotion is accomplished by the rotation of the plate-like colony around its shorter axis, the direction of rotation being to the right in some individuals and to the left in others. Pfeffer ('84), on the other hand, describes the rotation during the forward movement as alternately from the right and the left. Migula ('90) calls attention to the wavering, often backward, and irregular movements of this genus, and also notes its rotation about an axis through the middle of the colony. This rotation is either to the right or to the left, no predominance being mentioned. Polarity is thus marked in the activity of the *Gonium* colony, though not expressly marked in its structure except as it appertains to the individual cells.

In *Stephanosphæra* the polarity in structure is but slightly marked in the colony, being indicated in some colonies by the asymmetrical position of the cells, but there is a physiological differentiation in that one pole of the colony leads in locomotion. In this genus also, according to Cohn ('52), the rotation is in either of the two directions and is subject to frequent change. No predominant direction was noted by him.

In *Pandorina* the only structural expression of polarity is found in the greater development of the stigmata in the cells in the anterior end of the colony. In other particulars the poles are not differentiated. Braun ('51) maintains that in this genus the rotation is constantly around the long axis of the colony in the direction of the hands of a clock, when the motion is toward the observer. Nägeli (*fide* Bütschli, '83–'87, p. 858), on the other hand, observed rotation in both directions. My own observations upon *Pandorina morum* show beyond question that the direction of rotation is not constant, as the following table demonstrates.

Direction......	1	2	3	4	5	6	7	8	9	10	Total
Right over to left.	6+	12+	5±	1−	2±	3±	7±	1+	1+	3±	41
Left over to right.	6−	12−	4±	2+	2±	4±	6±	2−	1−	3±	42

The table gives the direction and number of changes in direction in rotation of ten colonies, each observed for one minute. The plus and minus signs indicate the estimated predominance in duration. According to the table the instances of direction observed are approximately equal for the two directions, though that from right over to left showed the greater duration. The younger and smaller colonies showed much the greatest activity and exhibited more frequent changes in direction than the older colonies. In all observed cases the same end continues to lead in locomotion, physiological polarity being thus fully developed in this genus.

In the case of *Eudorina* the structural polarity of the vegetative colonies is no more marked than it is in *Pandorina*, though according to Carter ('58) there is in the monœcious sexual colony a differentiation, in that the four cells at one pole divide to form spermatozoa, while the remaining twenty-eight become egg-cells. It should be noted in this connection that no such colonies were observed by Goroschankin ('75) in the sexual generation. The literature at hand presents no precise statement as to locomotion in this genus. As observed by me, it closely resembles that described above for *Pleodorina illinoisensis*; viz., rotation around the long axis of the colony, the same pole constantly leading in progression. The direction of rotation is frequently reversed, though it was predominantly from right over to left in the cases observed. A functional polarity thus exists in this genus.

In *Volvox*, according to Klein ('90), there is a polar differentiation as regards the stigmata that is even more marked than it is in the genera previously mentioned. He finds that the cells of the pole directed forward in locomotion each possess a stigma which is especially large and intensely colored; that the color fades out and the stigmata become smaller and paler as the equator is approached; and that beyond this they are usually represented merely by a colorless oil-drop, which in some cases may even disappear. The posterior hemisphere is also marked by the development there of the gonidia, as was first shown by Cohn ('56), and occasionally ellipsoidal colonies are found whose long axis connects the anterior and posterior poles. Locomotion in *Volvox* is accomplished, as elsewhere in the family, by the rotation of the colony about its principal axis. Wills ('80) observed the predominance of the rotation to the right and its occasional brief reversal. Klein ('89) states that this preference is found in *V. globator*, but that it is not shown by *V. aureus*. In this latter species the changes are frequent and are often separated by a brief pause. Backward motion is rarely seen and lasts but a short time. In the case of *Volvox* the axis of rotation is slightly oblique, the center of the colony remaining in the line of progress, but the axis of rotation being inclined from above the line at the anterior pole to below it at the posterior one.

We thus find that *Pleodorina illinoisensis*, which exhibits both a structural and physiological polarity, shares with most, if not all, of the genera of the family to which it belongs, the physiological differentiation which is expressed in locomotion, and also, in observed cases, exemplifies the extreme form of a predominance of rotation in one direction.

We also find that the structural differentiation shown in the decadence of its posterior stigmata obtains in varying degrees in the other spherical and ellipsoidal genera of the family—least in *Pandorina*, most in *Volvox*. The genus *Pleodorina* agrees with *Volvox* in having a structural polarity based upon the division of the colony into vegetative and gonidial regions, but the differentiation is simpler. Of the two species of *Pleodorina*, the one here described exhibits the simplest possible differentiation of the colony consistent with the symmetry of the organism; viz., the differentiation of the anterior polar circle of four cells as vegetative members of the colony. Of the two species of the genus it thus stands nearer *Eudorina*, while its sister species *P. californica* approaches more closely to *Volvox* both in the number of cells and in the extent of the differentiation.

The discovery of this additional species of the genus *Pleodorina* thus supports the opinion expressed by Shaw ('94), who founded the genus, that it was intermediate between *Eudorina* and *Volvox* but nearer the former. Judging merely from the asexual stage, *P. illinoisensis* affords additional evidence of the close relationship of *Pleodorina* and *Eudorina*.

Throughout the preparation of this paper the writer has had constantly in mind the possibility that the form here described is merely a stage in the life cycle of *Eudorina*. A number of facts lend support to this hypothesis: (1) the occurrence of *Pleodorina illinoisensis* with *Eudorina elegans*; (2) their marked similarity, aside from the four vegetative cells, in structure and measurements; (3) the impossibility of separating the youngest free-swimming colonies of the two forms; (4) a considerable variation in the size of the vegetative cells in *Pleodorina*, grading toward the condition in *Eudorina*; (5) some evidence that in certain cases at least the vegetative cells may divide, one case of a 2-cell stage having been seen in the hundreds, if not thousands, of specimens examined, and one instance noted in which a maternal colony containing thirty-two daughter colonies had at one pole four colonies which were slightly smaller than the remaining twenty-eight; and (6) the occurrence of pleomorphism in the family *Volvocineæ*, Klein ('89 and '90) citing no less than twenty-four "combinations" in the case of *Volvox aureus*. It may then be that the form here described as *Pleodorina illinoisensis* is only a "*Pleodorina* stage" of *Eudorina*.

The abrupt disappearance of this supposed new species from the plankton prevented the carrying out of breeding experiments designed to test its validity, and it seems that the matter must remain undecided for the present. In the absence of satisfactory proof that the form here described is but a phase of the life cycle of *Eudorina* it has seemed best to the writer to make the above suggestion and to take the only course open in publication, namely, the description of the form as a new species, inviting the criticism of subsequent investigation. The dilemma here presented is by no means an isolated one in plankton work, nor is it new to the family *Volvocineæ*: witness the long confusion which existed over the two species of *Volvox*, *aureus* and *globator*, which has been at last cleared up by the excellent work of Klein ('89, '89a, '90) and Overton ('89). Another instance is often presented when *Pandorina* and *Eudorina* both occur in the same collections and the plankton statistician must decide to which genus each specimen observed must be referred. Typical specimens of each can be found, but all individuals do not conform to the type, or they may present conditions in which the conformation is obscured by some phase of the life cycle.

The asexual reproduction of *Pleodorina illinoisensis* (Pl. XXXVII.) resembles that of other species of the genus in that it is accomplished by the repeated division of the gonidial cells, resulting in the formation of daughter colonies in the maternal matrix. These escape later from the parent organism, and by growth attain the adult condition with the differentiation of the four vegetative cells. Five successive cell divisions, pervading all the cells of the parent organism except the vegetative cells, are necessary for the completion of the process, and result in the 2-, 4-, 8-, 16-, and 32-cell stages of the forming colonies. The first two of these divisions result in the formation of a quadrangular plate of cells—a form which is retained through the two succeeding divisions, which produce the 8- and 16-cell stages. The cupping of this plate, which results in the formation of an ellipsoidal colony, is apparent as early as the 4-cell stage (Pl. XXXVII., Fig. 9) and continues through the later stages (Fig. 11, 13), so that by the time the 16-cell plate is formed it has almost the curvature of a saucer. With the formation of thirty-two cells the closure of the cup proceeds and is soon completed. The orifice of the cup is directed outward in all cases, and thus the ends of the cells of the daughter colony which are formed from the *outer* end of the maternal gonidial cell come to lie in the inner side of the cup, and are the *inner* ends of the cells of the daughter colony. In the matured colonies the young usually lie with their long axes parallel to the surface of the parent. I have not, however, been able to identify the point of closure of this cup with this region or positively with any other.

The sequence and position of cleavage planes which produce the quadrangular plate of the 16-cell stage is, in the main, similar to that described by Goroschankin ('75) and Braun ('75) for *Eudorina* and *Volvox*. Beyond this stage there is some doubt as to the agreement. A full discussion of the subject is beyond the scope of the present paper, for which the following brief description must suffice. The first cleavage plane (I) divides the gonidial cell into two hemispheres along the axis of the cell, and the daughter nuclei, with the surrounding protoplasm, are placed close together in the center of the opposing faces of the new cells (Pl. XXXVII., Fig. 7). The second plane (II) is at right angles (Fig. 8–9) to the first and also passes through the regions representing the axis of the ancestral cell. In this instance also the nuclei are gathered near the center of the young colony, which exhibits to an appreciable extent the curving indicative of the later formation of the cup. The 8-cell stage results from the divisions of each of the quadrants of the 4-cell stage by a plane (III) which is parallel to one of the previous planes and perpendicular to the other, meeting the latter at a point about midway between the center and the circumference. By a subsequent adjustment of the cells the four more centrally placed ones come to form a sort of a Greek cross whose angles are filled by the other four (Pl. XXXVII., Fig. 10, 11). The 16-cell stage is formed by four additional planes, each of which divides one of the cross-cells and its corner neighbor. The location of these planes may be described in the same terms as the last excepting that they meet the radial planes, I and II, at about one fourth the distance from the circumference toward the center. The cupping of the plate soon advances to such an extent that it consists of a square of four centrally placed cells, upon each of the four sides of which there overhangs a row of three cells, of three grades of elevation (Pl. XXXVII., Fig. 12, 13). The succeeding division and the completion of the cup (Pl. XXXVII., Fig. 14) result in the young colony's assuming the ancestral form. Throughout these divisions the number of pyrenoids in the daughter cells grows steadily less. But one can be found in each cell in the 32-cell stage, while in the 16-cell stage two are readily recognizable in each cell. In the earlier stages and before division the number often varies, and the pyrenoids are frequently so crowded that enumeration is difficult if not impossible. It seems not improbable that these structures also must undergo some division during the process of cell multiplication. During the processes of division the nuclei continue to occupy a position near the inner ends of the cells (in the new colony), and it is only after the divisions have been completed that they come to occupy their usual positions at the center of the cells—perhaps as a result of the growth of the chromatophore.

No stage of sexual reproduction has been positively identified for this species.

A peculiar condition of the colonies of this species, also occurring in *Pandorina* and *Eudorina*, deserves passing notice. It occurred with considerable frequency in all three genera and resulted in each case in the destruction of the entire colony affected. The early stages of the disease, if it be such, are indicated by the homogeneous condition of the cells and the fading out of the color, together with a flattening of the cell contents into a disk- or lozenge-shaped mass (Pl.

XXXVI., Fig. 4). In the subsequent stages this mass assumes a yellow and then a brownish color, takes on an irregular shape (Pl. XXXVI., Fig. 5), and disintegrates, leaving the empty cell walls occupying the matrix. In spite of the suggestion in the above description there was never any trace of the formation of spermatozoa in the colonies presenting these phenomena, neither was there any indication of encystment. There was no indication of either a fungous or an algal parasite, and it seems not improbable that the occurrence of these diseased forms may have been due to some unfavorable local condition in the water tributary to the habitat of the genera affected.

The following brief synopsis of the prominent characters of this genus and its two species will serve as a convenient diagnosis for their determination.

Pleodorina Shaw.—Colony consists of a spherical or elliptical cœnobium of greenish biflagellate cells of two types, vegetative and gonidial, in the anterior and posterior parts of the colony respectively, which lie in the periphery of a hyaline gelatinous matrix and are surrounded by a common hyaline envelope. Cells each with one reddish stigma, which is more prominent in the anterior part of the colony. No connecting filaments between the cells. Non-sexual reproduction by gonidia, which are formed by increase in size of a part of the cells of the colony. Daughters escape from parent as small colonies of biflagellate cells which at this stage are all similar. Sexual reproduction not known.

P. californica Shaw.—Number of cells in colony 64 or 128. Maximum diameter of colony 175–340 μ. Vegetative cells constituting approximately one half the colony. Gonidial cells 2–3 times diameter of vegetative cells. Known habitat: ponds, ditches, and streams in California, Indiana, and Illinois.

P. illinoisensis n. sp.—Number of cells in colony usually 32, rarely 16 or 64. Dimensions of colony range from 46×38 μ to 200×175 μ. Vegetative cells always four in number. Gonidial cells approximately 1.1—2 times diameter of vegetative cells. Known habitat: submerged lands along the Illinois River. Types deposited in collections of Illinois State Laboratory of Natural History and United States National Museum.

Illinois Biological Station, Havana, Ill., July 25, 1898.

BIBLIOGRAPHY.

Braun, A.

'51. Betrachtungen über die Erscheinung der Verjüngung in der Natur, insbesondere in der Lebens- und Bildungsgeschichte der Pflanze. XVI+364 pp., 3 Taf. Leipzig.

'75. Über einige Volvocineen. Ber. d. Gesellsch. naturforsch. Freunde zu Berlin, 1875, pp. 9–18.

Bütschli, O.

'80–'89. Protozoa. Bronn's Klassen und Ordnungen des Thierreichs, Bd. I., Th. I.–III. 2035 pp., 79 Taf. Leipzig und Heidelberg.

Carter, H. J.

'58. On Fecundation in Eudorina elegans and Cryptoglena. Ann. Mag. Nat. Hist., Ser. 3, Vol. II., pp. 237–253, Pl. VIII.

Clinton, G. P.

'94. Pleodorina in Illinois. Botan. Gazette, Vol. XIX., p. 383.

Cohn, F.

'52. Über eine neue Gattung aus der Familie der Volvocineen. Zeitschr. f. wiss. Zool., Bd. IV., pp. 77–116, Taf. VI.

'56. Observations sur les Volvocinées. Ann. d. Sci. Nat., Botan., IV. Ser., T. V., pp. 323–332; also, with some changes, in 34 Jahresber. d. Schles. Gesellsch. f. vaterl. Cultur f. 1856, pp. 39, 40, 77–83.

Fresenius, G.

'56. Über die Algengattungen Pandorina, Gonium und Raphidium. Abhandl. der Senckenberg. naturforsch. Gesellsch., Bd. II., pp. 187–200, Taf. VIII.

Goroschankin, J.

'75. Genesis im Typus der palmellenartigen Algen. Versuch einer vergleichenden Morphologie der Familie der Volvocinea (German title of Russian article). Mittheil. der kais. Gesellsch. naturforsch. Freunde in Moskau, Bd. XVI. 40 pp., 2 Taf.

Henfrey, A.

'56. Notes on some Fresh-water Confervoid Algæ New to Britain. Trans. Micr. Soc. London, N. S., Vol. IV., pp. 49–54, Pl. IV.

Klebs, G.

'86. Über die Organisation der Gallerte bei einigen Algen und Flagellaten. Untersuch. a. d. botan. Inst. zu Tübingen, Bd II., pp. 333–418, Taf. III., IV.

Klein, L.

'89. Morphologische und biologische Studien über die Gattung Volvox. Pringsheim's Jahrb. f. wiss. Botan., Bd. XX., pp. 133–211, Taf. X.—XII.

'89a. Neue Beiträge zur Kenntniss der Gattung Volvox. Ber. d. deutsch. botan. Gesellsch., Jahrg. 1889, Bd. VII., pp. 42–53, Taf. III.

'90. Vergleichende Untersuchungen über Morphologie und Biologie der Fortpflanzung bei der Gattung Volvox. Ber. d. naturf. Gesellsch. zu Freiburg i. B., Bd. V., pp. 29–120, Taf. II.—VI.

Migula, W.

'90. Beiträge zur Kenntniss des Gonium pectorale. Botan. Centralbl., Bd. XLIV., pp. 72–76, 103–107, 143–146, Taf. II.

Mottier, D. M.

'94. Pleodorina in Indiana. Botan. Gazette, Vol. XIX., p. 383.

Overton, E.

'89. Beitrag zur Kenntniss der Gattung Volvox. Botan. Centralbl., Bd. XXXIX., pp. 65–72, 113–118, 145–150, 177–182, 209–214, 241–246, 273–279, Taf. I.–III.

Pfeffer, W.

'84. Locomotorische Richtungsbewegungen durch chemische Reize. Untersuch. a. d. botan. Inst. zu Tübingen, Bd. I., pp. 363–482.

Shaw, W. R.

'94. Pleodorina, a new genus of the Volvocineæ. Botan. Gazette, Vol. XIX., pp. 279–283, Pl. XXVII.

Wills, A. W.

'80. On the Structure and Life History of Volvox globator. Midland Naturalist, Vol. III., pp. 209–214, 233–237, Pl. VII., VIII.

EXPLANATION OF PLATES.*

ABBREVIATIONS.

A.,	anterior pole.	*ncl.*,	nucleolus.
chr.,	chromatophore.	*o. l.*,	outer layer of sheath.
c. m.,	cell membrane.	*P.*,	posterior pole.
f.,	flagellum.	*p. c.*,	protoplasmic column.
fu.,	furrow.	*pr.*,	pyrenoid.
g. c.,	gonidial cell.	*r.*,	reticulum.
i. l.	inner layer of sheath.	*s.*,	stigma.
m.,	matrix.	*sh.*,	sheath.
m. m.,	matrix membrane.	*v. c.*,	vegetative cell.
n.,	nucleus.	I, II, III,	cleavage planes.

Plate XXXVI.

Fig. 1. *Pleodorina illinoisensis*, lateral view of colony. × 500.
Fig. 2. Lateral view of vegetative cell. × 1500.
Fig. 3. Lateral view of gonidial cell. × 1500.
Fig. 4. Lateral view of matured colony, showing posterior lobes. × 185.
Fig. 5. Diseased cell, early stage. × 1250.
Fig. 6. Diseased cell, later stage. × 1250.

Plate XXXVII.†

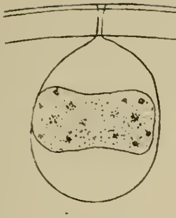
Fig. 7. *Pleodorina illinoisensis*, top view of 2-cell stage.
Fig. 8. Top view of 4-cell stage.
Fig. 9. Lateral view of 4-cell stage.
Fig. 10. Top view of 8-cell stage.
Fig. 11. Lateral view of 8-cell stage.
Fig. 12. Top view of 16-cell stage.
Fig. 13. Lateral view of 16-cell stage.
Fig. 14. Top view of 32-cell stage.

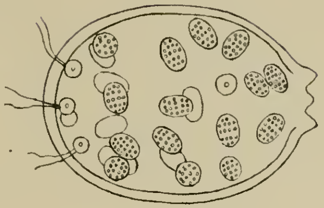
---

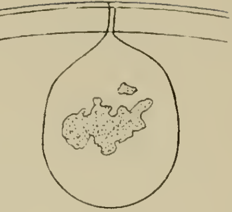
* Figures drawn by C. A. Kofoid and inked by Miss L. M. Hart.

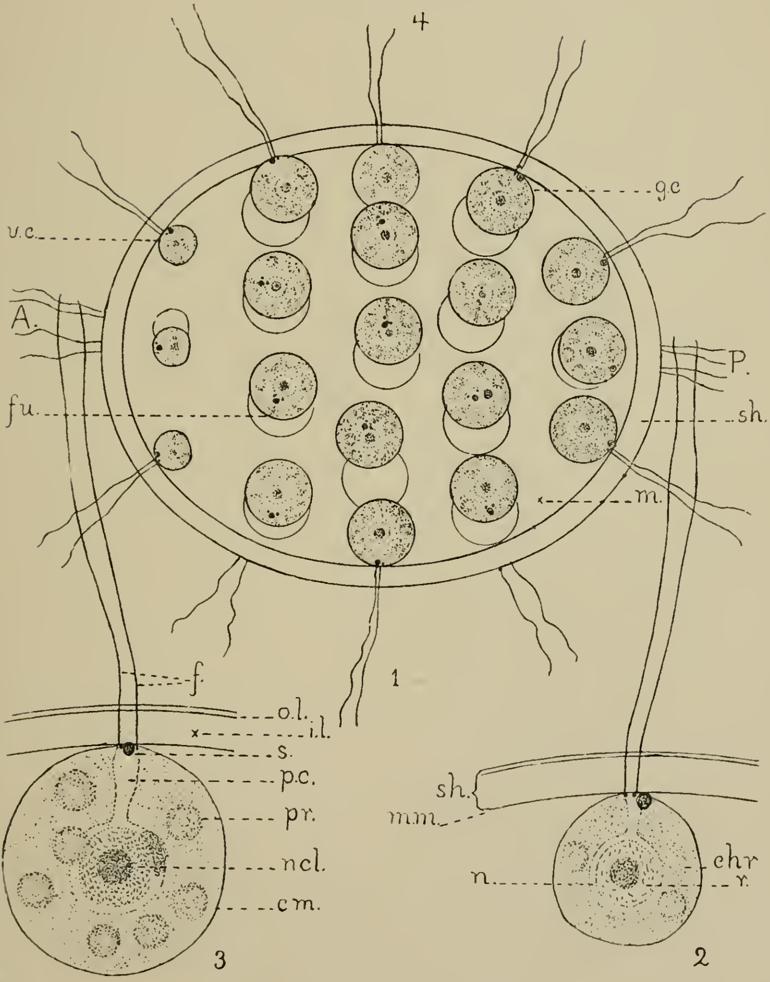
† Figs. 7-14 magnified 1000 diameters.

PLATE XXXVI.


5


4


6



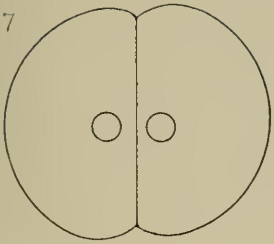
1

3

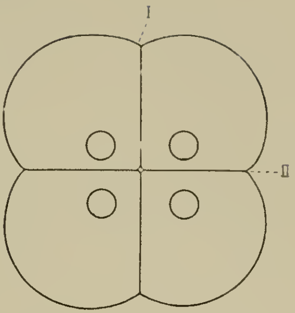
2

Plate XXXVII.

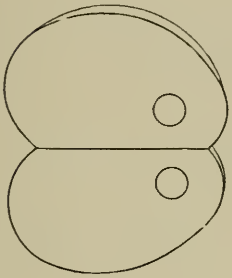
7



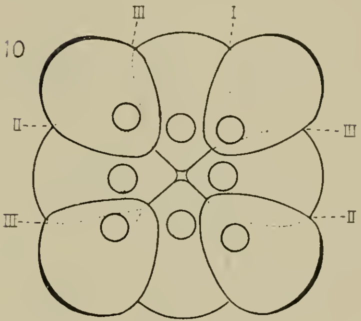
8



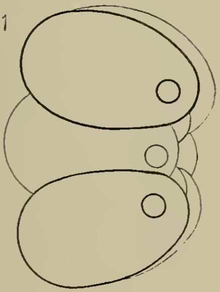
9



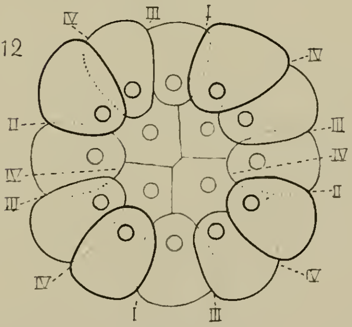
10



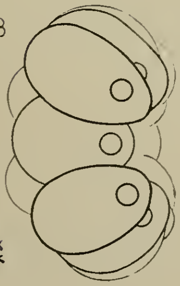
11



12



13



14