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CHRYSOCRINUS HONORARIUS SPEC. NOV. (STYLOCOCCACEAE, CHRYSOPHYCEAE); SOME STAGES OF ITS LORICAL DEVELOPMENT AND ITS DISTRIBUTION ON THE SUBSTRATE

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SUMMARY

Chrysocrinus honorarius spec. nov. is described from a moorland near Staverden, The Netherlands. The taxonomic position of the new taxon is discussed with special regard to *Chrysocrinus* spp. and *Kybotion globosum* (Matv.) Bourr. (Syn.: *Tylochrysis globosa* (Matv.) Matv.). SEM observations show that this new species exhibits a combination of characters of the two above-mentioned taxa. The manifestation of these characteristics appears to depend on the age of the specimen. The distribution of the different stages over different parts of submerged *Sphagnum* plants (its natural substrate) is described. The alga decidedly prefers as its habitat the chlorocytes of the outer surface of the apical parts of the *Sphagnum* leaves.

1. INTRODUCTION

During a survey of benthic Chrysophyceae in the Mid-Netherlands a taxon was encountered that possesed characteristics of two different genera as will be discussed below. Because of this ambiguity a more detailed study was necessary so as to obtain more information concerning this organism primarily to assess its possible systematic position.

The genus *Chrysocrinus* was erected by PASCHER (1916, pp. 104 sqq., p. 115, f. 11, T.8: 1–9) for his species *C. hydra*; later on PASCHER (1940) described another four species, viz., *C. irregularis* (p. 343, f. 5a–d), *C. gibbosus* (p. 343, f. 5h–i), *C. tubulosus* (p. 343, f. 5e–f) and *C. cyanophycearum* (p. 343, f. 5g). The sixth species, *C. polyedricus*, was described by CONRAD (1942, p. 36, f. 9). Apart from the original descriptions but few subsequent records of these species are encountered in literature (*C. tubulosus*: BOURRELLY 1957, pp. 311 sq., T. 10: 25–27; *C. irregularis*: CONRAD 1942, p. 36; ETTL 1968, pp. 214, 216, T. 7: 1–3, 10), and our cognisance of these organisms is, therefore, scanty. *Chrysocrinus (fig. 1)* is characterized by a hemispherical lorica, with its flat surface attached to the substrate and its exposed surface pierced by many small pores rendering the appearance of a colander. The ornamentation of the lorica and the configuration

† On September 26th 1983 Maarten Peter Schoonoord untimely died at the age of 23; his premature death left the second author with the sad task of finishing the manuscript alone.

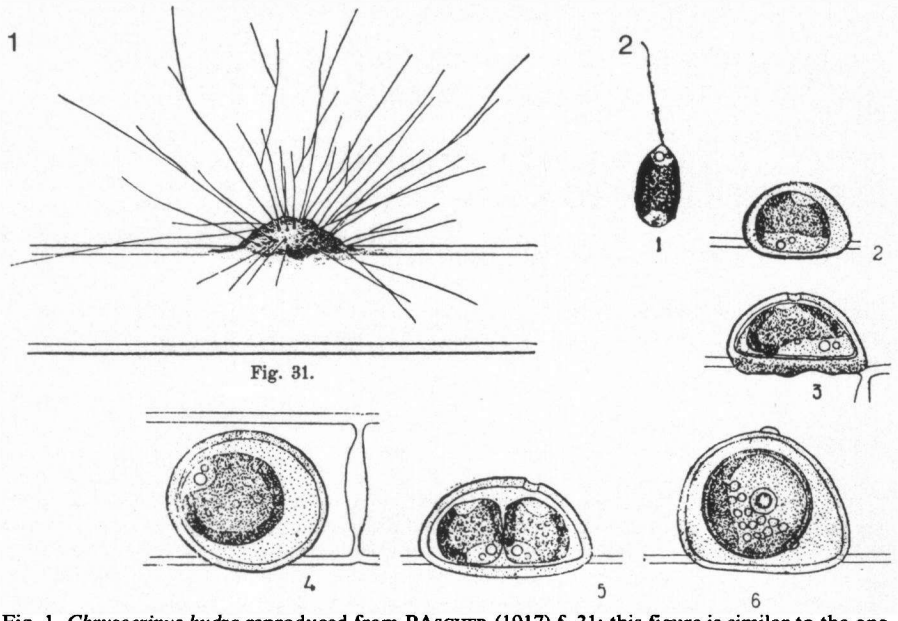


Fig. 1. *Chrysocrinus hydra* reproduced from PASCHER (1917) f. 31: this figure is similar to the one published with the original description (1916, T. 8:1) as a chromolithography.

Fig. 2. *Kybotion globosum* (*"Chrysotylos globosus"*) (MATVIENKO 1938, f. 1–6).

of the pores are of major importance for the distinction of the species. The lorica can be iron-encrusted and, if so, has a yellowish-brown colour and an irregularly thickened lorica wall. Within the lorica a small protoplast is present; this cell is globular to ellipsoid and sometimes possesses very fine, filiform rhizopodia extending through the pores of the lorica. These are used to capture small particles for phagocytotic feeding (PASCHER 1916, pp. 105, 115; 1917, p. 35). On account of its rhizopodial cells and its amoeboid mode of reproduction as described by PASCHER (1916, pp. 106, 115, T. 8: 2, 7; 1917, pp. 35 sq.), *C. hydra* is classified by BOURRELLY in the Order Rhizochrysidales (1957, pp. 311 sq.).

The second epiphytic taxon important in this context is *Kybotion globosum* (Matvienko) Bourrelly (*fig. 2*), in which species the cell is also enclosed in a hemispherical lorica but in contrast to *Chrysocrinus* there is only a single, large subapical pore instead of many small ones. This species, originally described under the name *Chrysotylos globosus* (MATVIENKO 1938, pp. 157 sq.; p. 160, f. 1–6), has repeatedly been subjected to name changes: Pascher, took it for a member of the genus *Lagynion* and changed the original name (as *"Chrysotilos globosus"* in error, see below) into the new combination *Lagynion globosum* (1949, p. 59).

Apparently unaware of this event, MATVIENKO himself (1952, p. 32) renamed it into *Tylochrysis globosus* without giving any reason, but most probably because the name *Chrysotylos* was preoccupied by the genus *Chrysotilos* (Chryso-

capsaceae) erected by PASCHER in 1931 (pp. 88 sqq, f. 11–18b) and in 1965 (p. 71) MATVIENKO changed the name to its correct grammatical form: *Tylochrysis globosa*. In his review on Chrysophyceae BOURRELLY (1957, p. 289) classified it under the genus *Kybotion* and made another new combination, viz., *Kybotion globosum* (Matv.) Bourr., this on the ground of the flagellate swarmers mentioned by MATVIENKO (1938, p. 160, f. 1), which excludes it from the genus *Lagynion*; in consequence it is now ranked under the Chromulinales (BOURRELLY 1957, p. 289). Although all three species classified in the genus *Kybotion* by Bourrelly fit in with the definition of the genus as to general cell structure and multiplication by means of swarmers, the inclusion of *K. globosum* renders it inhomogeneous because of the difference in the shape and particularly in the structure of the lorica wall of *K. globosum* as compared to other two species, not to mention the fact that it hardly ever forms filipodia (or not at all, because thus far nobody has observed any).

However, the scruples enumerated above are done away with by KRISTIANSEN's (1982, p. 84) rearrangement in which the two genera discussed above are classified in one and the same family, Stylococcaceae.

Of the specimens studied by us some matched the description of *Chrysocrinus* sp., whereas other ones fitted *K. globosum* more satisfactorily; it will be shown that this discrepancy is rather due to differences in age of the specimens than to their belonging to two different taxa.

2. LOCALITY AND HABITAT

The habitat, a small moorland, is situated near Staverden (Municipality of Ermelo, Province of Gelderland) and known as "De Leemputten". A description of this area was given by ELLIS-ADAM (1983). The chrysophycean taxon in question was exclusively found in shallow pits with, as dominant submerged vegetation, *Sphagnum crassicladum* var. *obesum* (Wils.) Jans. & Wacht. with some *S. cuspidatum* Hoffm., the pH ranging from 3.1 to 6.5 and a pH corrected conductivity (at 25°C) of 30 to 170 $\mu S.cm^{-1}$. It is the same site where *Chrysostephanosphaera hyalocytobia* as described by ELLIS-ADAM (1982) has been found. The algae can be found all the year round, even under an ice cover, but appear to reach their optimum abundance in early summer. Apart from the above mentioned locality it was recorded by the first author from a submerged *Sphagnum* sp. collected at the "Brunssummer Heide" (near Heerlen and Brunssum, Province of Limburg), a moorland in the southern part of The Netherlands, and also from several small fens in the Isle of Skye (Scotland). Conceivably this organism will turn out to be a rather common epiphyte on submerged peat mosses in moorlands. Our organism is possibly identical with certain unnamed specimens figured by PASCHER (in BREHM 1930, f. 59 and 1940, f. 2).

3. MATERIALS AND METHODS

Samples of *Sphagnum crassicladum* var. *obesum* and *S. cuspidatum* with the epi-

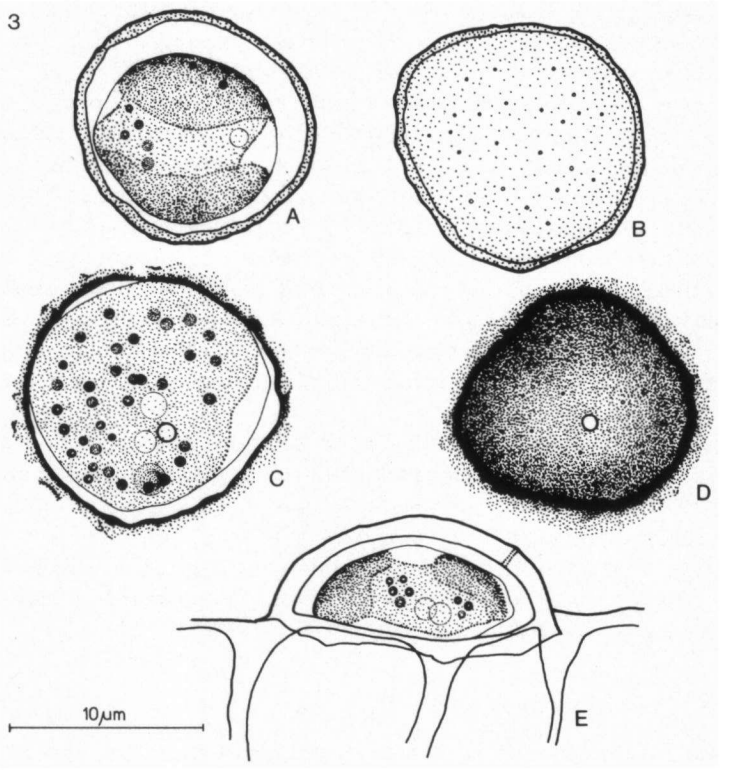
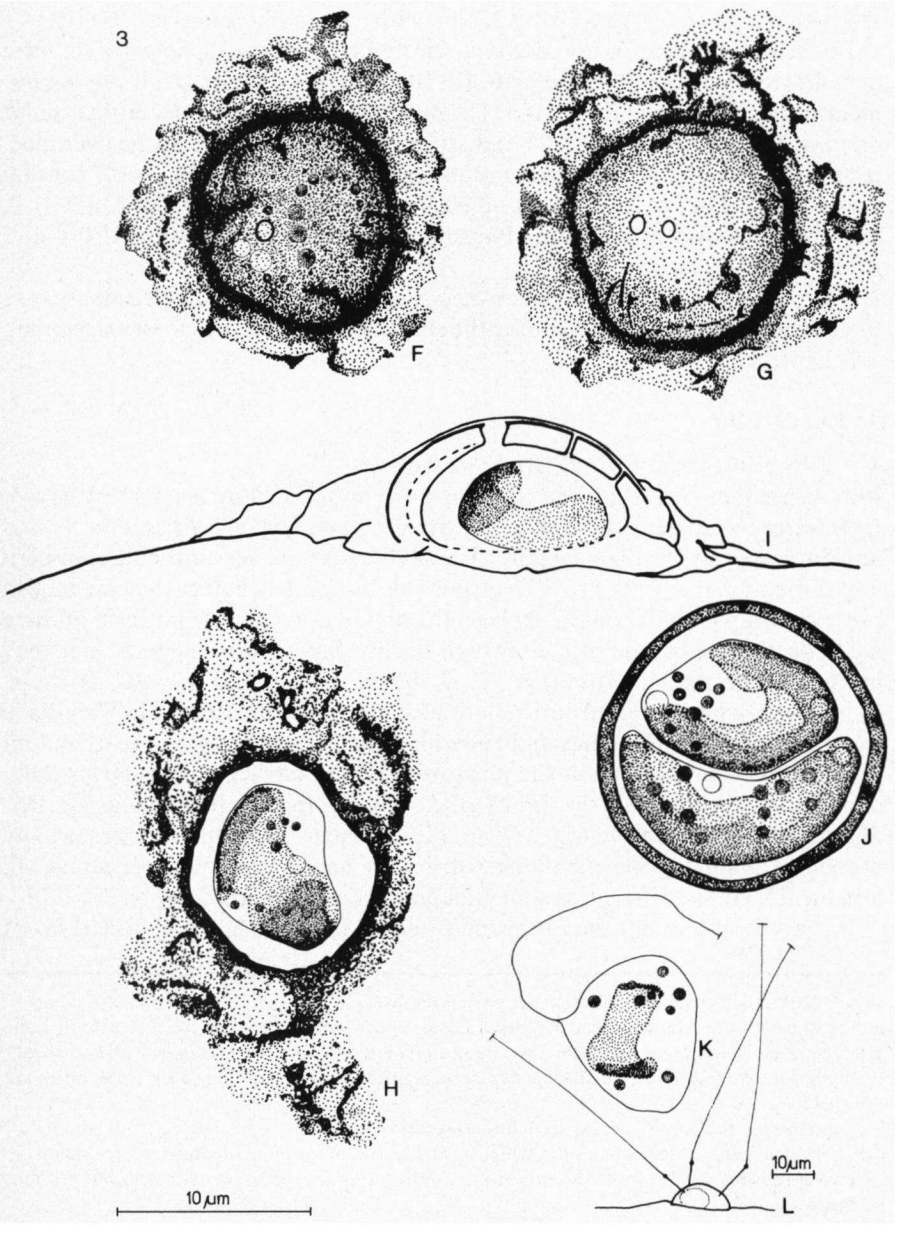


Fig. 3A–L. Drawings after photomicroscopical observations on *Chrysocrinus honorarius*.
A. A non-encrusted individual (apical view, optical section); the chromatophore, two pulsating vacuoles (shown as perfect circles of dots) and some leucosine droplets (all other dotted subglobose elements, the majority of which is appreciably smaller than the pulsating vacuoles) are visible.
B. Lorica of a similar specimen as in *fig. 3A* (apical view); the pores are sometimes seen as strongly light-diffracting dots.
C. An averagely incrusted specimen (apical view, optical section).
D. Lorica of a similar specimen as in *fig. 3C* (apical view); one large subapical pore is present.
E. A similar specimen as in *fig. 3A* (lateral view, optical section); a site of a small pore is indicated.
F. TYPE. Heavily encrusted specimen with one subapical pore and several small pores in the lorica; the cell contains one chromatophore, two pulsating vacuoles and several leucosine droplets (apical view).
G. Lorica of a similar specimen as in *fig. 3F*, but with two subapical pores (apical view).
H. Specimen with an exceptionally heavy iron cover (apical view, optical section); the iron layer covers some of the neighbouring substrate.
I. A similar specimen as in *fig. 3H* (lateral view, optical section); the subapical pore and two of the small pores are in focus.
J. Two similar, immobile cells in a lorica; possibly a reproductory stage; (apical view, optical section).
K. Flagellate cell; lorica not drawn.
L. A small perforated lorica with extruded rhizopodia; due to the insufficient magnification during its observation only a rough sketch could be made (lateral view).



phytic organisms were kept at 20 °C under a daily light/dark cycle of 8/16h.

All photomicroscopical observations and drawings were made of living material, on a scale of 1:2000 with the aid of a Zeiss RA microscope provided with a drawing tube and magnified twice before inking by means of a pantograph or drawn directly on a scale of 1:4000 using a 2 × magnifying extension. Photo-

graphs were taken on Agfa Ortho 25 film with a Zeiss Photomicroscope III.

For scanning electron microscopy, selected branches of *Sphagnum* sp. were dehydrated by a series of 20, 30, 50, 70, 100% ethanol, the material staying immersed in each fraction for 15 min. The material was subsequently critical-point dried in a Polaron E 3000 CPD and stuck to a specimen stub with silver glue. It was subsequently gold/palladium sputtered in a Polaron SEM "cool" coating unit E 5000. The material was studied by means of a Cambridge Mark II A Scanning Electron Microscope. Photographs were taken on Ilford FP 4 and Ilford Pan F.

The procedures followed for the counting of the number of specimens so as to determine their distribution over the substrate are described in the pertaining subchapters.

4. TAXONOMY

4.1. Photomicroscopical observations

Many specimens with hyaline (*fig. 4a*) or light yellow loricae were observed; their loricae were perforated by many minute pores (*fig. 3A, B*) and rarely had an additional, large subapical pore. The small pores are very difficult to discern and sometimes it did not prove to be possible to decide whether they are indeed pores or shiny globules on the surface. In conjunction with the position of these algae on their substrate (see elsewhere in this paper), this suggests that they are young specimens. More (*fig. 3F, G, H*; *4a, f, h*) or less (*fig. 3C, D*; *4c, d, e*) intensely iron-encrusted loricae are also regularly encountered. They have a brownish colour. Granular and cone-like structures (*fig. 4h*) are scattered on the lorica and in addition at the juncture with the substrate granular or flaky deposits may be present (*fig. 3F, G, H, I*; *4f, g, h*). In such loricae one (*fig. 3C, F, I*; *4a, d, f, h, i*) or more (*fig. 3G*; *4a*) (up to four) subapical pores are usually present. The small pores mentioned earlier are hardly discernible or not at all, and the thickness of the lorica sometimes has increased considerably.

It must be noted that the forms indicated as Type 3, 3A, 3B by ELLIS-ADAM

Fig. 4a–i. Photo micrographs of *Chrysocrinus honorarius*.

a. The algae on their substrate (apical view); the specimens growing on chlorocytes exhibit some granular deposits and large subapical pores (at the left hand side there is a specimen with two pores); the light dots around the bases of the loricae are ironhydroxide concretions also; small pores are not visible.

b. A specimen with a smooth transparent lorica (apical view).

c. A specimen with a lorica more tinged with iron and with some granular deposits (apical view).

d. Two specimens with an average rate of incrustation (apical view); one with and one without a subapical pore.

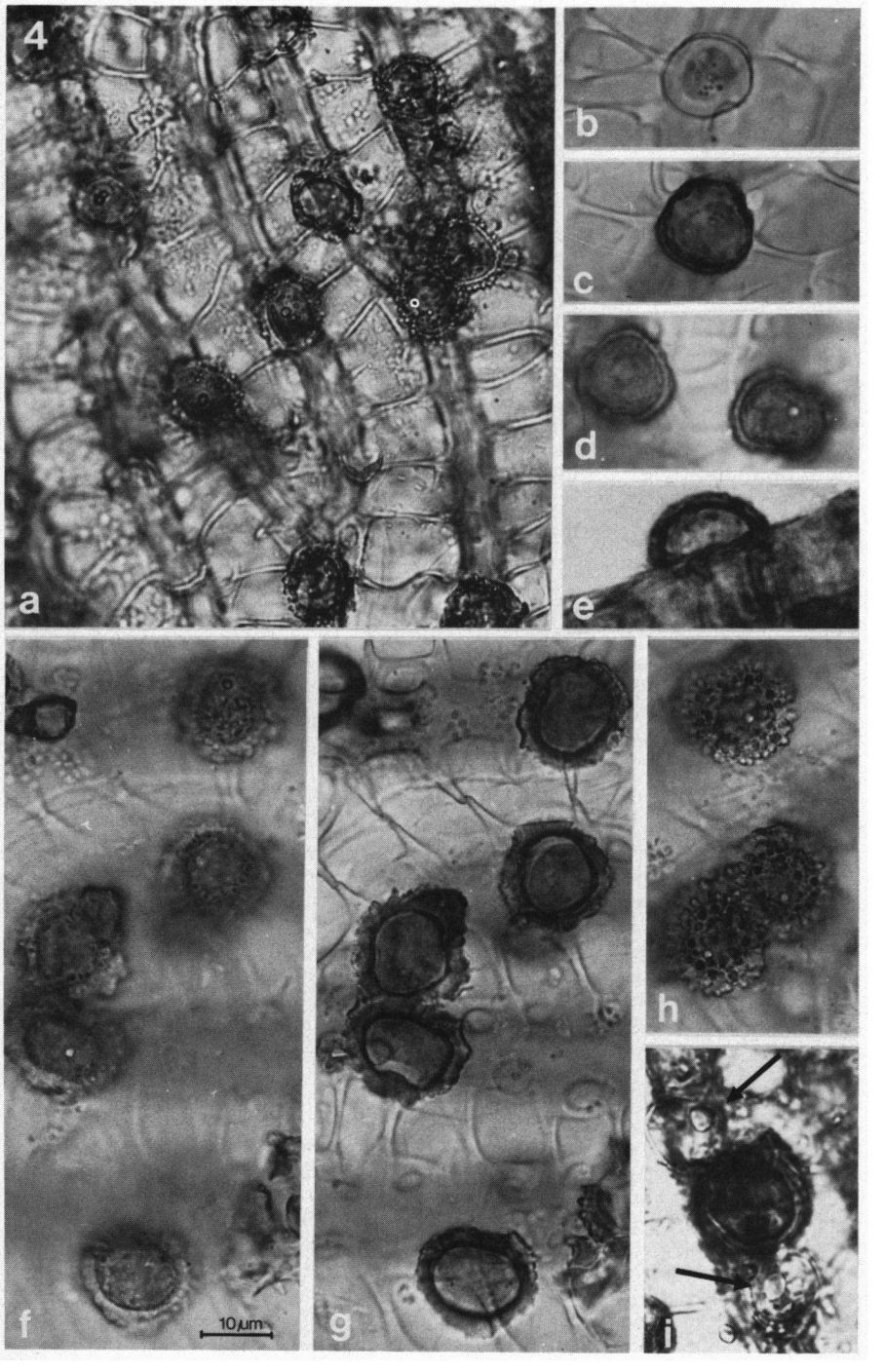
e. A specimen without a subapical pore (lateral view); small pores visible.

f. Heavily incrusted specimens (apical view); the subapical pores clearly visible.

g. The same specimens as in *fig. f* in lower focus, showing the cells and the basal incrustation.

h. Loricae with cone-wise structured deposits on their surface (apical view).

i. Two loricae with large holes (arrows) (apical view); the lorical surface is smooth and transparant; apart from an unidentified specimen of a different epiphyte, another specimen of the species under discussion visible and shows its considerably smaller subapical pore.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(1983, p. 2, table 2a) belong to the form described above.

The cell enclosed in the lorica contains a large, yellowish brown chromatophore without a stigma. Also small and often numerous leucosine droplets and one to three pulsating vacuoles (*fig. 3A, C, E, F, H*; *4b, c, g*) could be observed in many cells. The observation of cells within dark coloured loricae is very cumbersome, however. Although one may expect that the different types of orifices in the lorica serve to project rhizopodia through them, one becomes disappointed in this respect. Only once did the second author catch a glimpse of a specimen contained in a lorica with only small pores that had extruded rhizopodia of a most unusual type. Each rhizopodium consisted of a stiff basal part ending in a minute globule bearing a very long tenuous extension, the globule apparently functioning as a kind of ball-head allowing the thin part to take up different positions, the movements being either gradual and slow or quick and shockwise as if a triggering mechanism had started it. Unfortunately this observation was made at an insufficient magnification and only a rough sketch could be made (*fig. 3L*). On several occasions loricae were encountered that contained two cells (*fig. 3J*). A similar observation was made by PASCHER (1916, p. 106). Unfortunately the fate of such cells could not be determined, but most probably represent some reproductory stage. On one occasion a flagellate cell (*fig. 3K*) was observed in a lorica by the first author; it was slightly metabolic and contained a green strap shaped chromatophore and some minute leucosine droplets.

A curious phenomenon was seen to be present in empty loricae: they appeared to possess a large apical or subapical hole of about 3μm in diameter (*fig. 4i*). This can be thought of as having served as an exit for reproductory cells.

Fig. 5a–i. SEM micrographs of *Chrysocrinus honorarius*. Scale bar in a, b, c, d, e: 10 μm; in c, f, g, h, i: 1 μm.

a. An overall view of a part of a *Sphagnum* leaf with epiphytes, the smooth chlorocytes on which the algae are attached and the humped hyalocytes clearly recognisable. The density of the epiphytes, and the fact that no other algal species are present are striking.

b. Two young non-encrusted specimens growing on *Sphagnum crassicladum* var. *obesum*. The zip fastener like structures running to the bottom of the picture represent the thickened rings on the inner sides of the hyalocytes.

c. Magnification of one of the specimens of *fig. 5b*; the small pores in the lorica visible.

d. Specimens encrusted with iron; small grains at the base of the lorica visible; in one of the loricae two pores present.

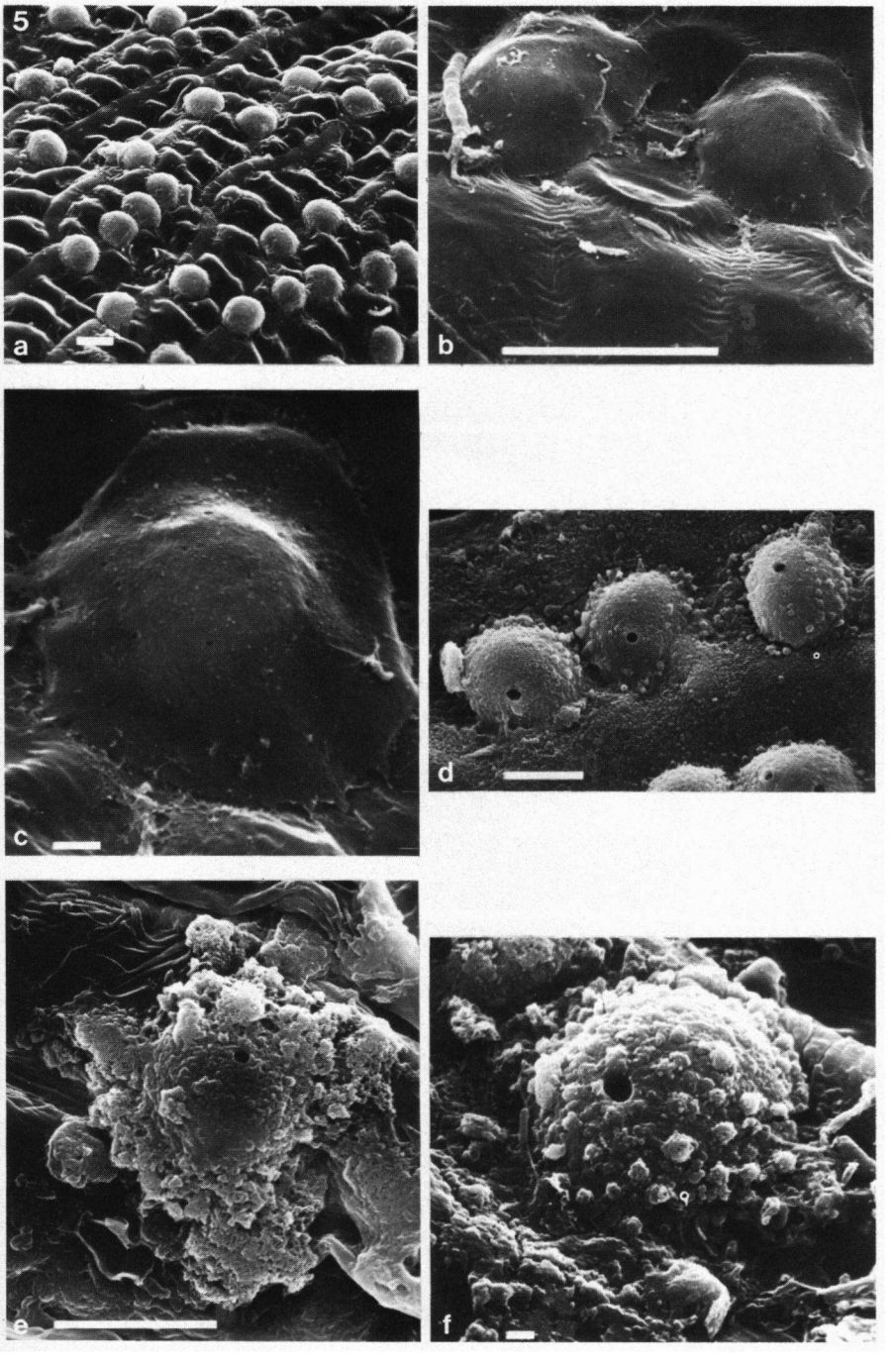
e. Lorica with extremely heavy iron cover in which, apart from the subapical pore, no distinct structure is visible. The cover extends over a large portion of the substrate.

f. An example of a richly structured iron deposit; small iron pellets present in which a small pore is readily visible; also the large pore can be seen.

g. Two specimens with comparatively smooth loricae; one with a completely formed subapical pore, one in which the pore is still in a phase of development. The surface of the chlorocyte runs into the left bottom corner and two hyalocyte humps are at the bottom of the picture.

h. A lorica, presumably without a protoplast, with a hole (see text for explanation). It must be noted that here also small pores are present. Subapical pore lacking.

i. A stage of formation of a hole is shown, which makes it clear why the small, original subapical pore disappears when the hole is formed.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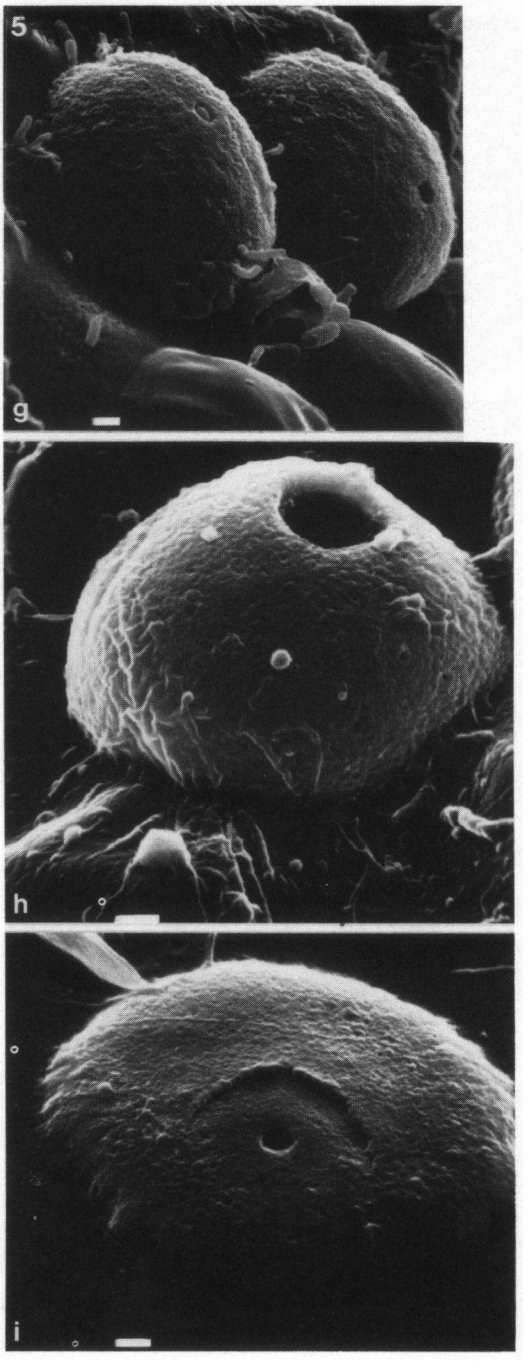
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4.2. Scanning electron microscopy

Observations made with SEM techniques confirm much of the above mentioned features and in addition gave some answers to questions not solvable by means of light microscopy. The photographs will not all be discussed in great detail in the text so as to prevent duplication of the legends.

The non-encrusted individuals have a flat, pancake shaped lorica with small pores (*fig. 5b, c*). The specimens with rather large subapical pores are always more or less strongly encrusted (*fig. 5d, e, f*); the presence of more than one pore is shown in *fig. 5d*. In addition small pores can be observed in individuals that are not heavily encrusted. By studying the iron deposit its influence on the small pores can be assessed. One can observe that the small pores indeed become reduced in size but not quite clogged by the iron encrustation. In some cases the deposit forms a collar around the pore, a minute channel through the lorica remaining visible as a pore (*fig. 5f*).

This local, heavier deposit of iron might possibly by explained as follows: oxygen liberated as a result of the photosynthesis activity of the protoplast leaks out of the lorical space through the pores to form a relatively high concentration in their vicinity, dissolved iron thus becoming oxydized and precipitating especially near those sites.

Since the different types were assumed to belong to the same species the formation of the subapical pore (*fig. 5g*) and the larger hole (*fig. 5h, i*) had to be demonstrated.

4.3. Discussion

The alga under discussion is thus characterized by two major properties of the lorica which can also be found, but separately, in the two genera mentioned earlier: many minute pores and a large subapical pore, characterizing *Chrysocrinus* spp. and *Kybotion globosum*, respectively. The hardly and the heavily (i.e., the younger and the older) encrusted individuals possessing small pores only would, therefore be referable to *Chrysocrinus*. In shape and dimensions our species resembles *C. hydra* most but we do not believe it to be identical as, according to PASCHER's description (1916, p. 105; 1917, p. 35), *C. hydra* is covered by a "thicket" ("einem ganzen Wald" and "mit einem Walde", respectively) of branched rhizopodia, which does not hold for our specimens. The specimens bearing large pores differ from *K. globosum* mainly in their smaller size (they are up to 8 μm smaller in diameter). The fact that in its original description (MATVIENKO 1938, p. 160) only one pore is mentioned is of little significance because only on rare occasions is there more than one pore present. The same also holds for Matvienko's lack of mentioning the presence of small pores: they really are very hard to discern and to interpret by light microscopy alone, so that one can well imagine that he overlooked them.

As regards the mode of reproduction, PASCHER (1916, p. 106) observed amoeboids in *Chrysocrinus hydra* and MATVIENKO (1938, p. 160, f, 1) flagellates in *Kybotion globosum*. Our attempts to culture the organism have failed thus far. Our observation of a flagellate does not preclude that this organism would be

able to form amoeboid reproduction cells also, several Chrysophyceans being known to have either kinds of reproductory cells. The possibility that they represent the asexual and the sexual stages of the cycle should also be kept in mind. In view of the above mentioned data we decided to describe our organism provisionally as a new species of the genus *Chrysocrinus*, because the minute pores are present during its entire lifetime. Another – albeit in this context a minor – consideration is that *Chrysocrinus* takes priotity over *Kybotion*. Since neither of us has ever seen any material of *K. globosum*, we think it premature to transfer this species to *Chrysocrinus*, thus leaving open the possibility that indeed it has no small pores and may be an independent taxon after all. If eventually it turns out that *K. globosum* has small pores also, then the time will have come to transfer it to *Chrysocrinus* or even – if there would be any cogent reason to do so – to unite it with our species, possibly in the genus *Tylochrysis* to be reinstated. For the time being we prefer to leave it in *Kybotion*.

4.4. Description

This species is dedicated to Prof. dr. A. D. J. Meeuse on the occasion of his retirement.

Chrysocrinus honorarius Schoonoord & Ellis-Adam, n.sp.

Lorica hemisphaerica substrato facie applanata adhaerens plerumque altitudine dimidio minore quam diametro, ordinatim 5–7 μm ac 7–16 μm variante, conspecta e vertice circumscriptione plerumque circulare sed etiam ellipsoidea interdum leviter reniforme quoque hyalina vel colore flavescente ad ferruginea tincta facie exteriore laeve vel depositione ferrata ad 8 μm irregulariter incrassata. Paries loricae porulis minutulis diametro minore quam 0.5 μm temere sparsis ac insuper plerumque speciminibus incrustatis poro unico subapicale nonnumquam poris pluribus, diametro circiter 1 μm, munitus.

Cellula loricam non replens, diametro variante 7–12.5 μm, non solum uno magno chromatophoro parietale lutescente-ochraceo stigmate privo sed etiam duabus vacuolis pulsantibus atque multis guttulis leucosini instructa.

Typus: figura nostra 3F.

Cellulis chlorophylliferis generis Sphagni aliquarum specierum submersarum habitat.

Lorica hemispheric, generally half as high as wide, attached to the substrate with its flat surface. Outline of the lorica in apical view mostly circular to sometimes ellipsoid, occasionally slightly reniform. The diameter of the lorica ranges from 7–16 μm, the height from 5–7 μm. The lorica colour varies from hyaline to yellow and brown, the surface from smooth to irregularly thickened by iron-encrustation, the iron layer may be up to 8 μm thick. The lorica wall is provided with many, randomly scattered minute pores less than 0.5 μm in diameter; in addition in encrusted individuals a single subapical pore, sometimes more than one, with a diameter of approximately 1 μm, usually present.

The protoplast does not fill the lorica completely, its diameter ranging from

7–12.5 μm. The cell contains one large parietal yellowish-brown chromatophore without a stigma, two pulsating vacuoles and many small leucosine droplets.

Type: *fig. 3F.*

Habitat: growing on chlorocytes of submerged *Sphagnum* species.

We realize that the dimensions mentioned above seemingly disagree; this is due to the impossibility to record all dimensions of every specimen satisfactorily.

5. Distribution on the substrate

At first sight one gets two impressions, viz., that the apical half of the *Sphagnum* leaf is more densely occupied than the basal one and that the *Chrysocrinus* specimens are mainly found on chlorocytes (*fig. 4a*) (which is not exceptional as it was already mentioned by BREHM (1930, p. 168, f. 58) based on data supplied to him by Pascher and later figured by PASCHER himself (1940, f. 2, T. 11: 1)). A possible explanation may be that the depressions formed by the chlorocyte/hyalocyte configuration are relatively sheltered (*fig. 5a*) and, therefore, more favourably situated for the settling of motile reproductive cells. In addition the protoplast might extract metabolites from the chlorocyte; it is doubtful, however, whether those substances are of vital importance, since we found our organisms also growing on artificial substrates viz., microscope slides. The observation that the tips of the *Sphagnum* leaves look more densely occupied than their bases may partly be explained by the difference in chlorocyte/hyalocyte ratio; this ratio being larger (and thus providing a relatively greater chlorocyte surface area available as a substrate) at the tip and smaller at the base of the leaf. However, a more important factor explaining this phenomenon seems to be the preference of the epiphytes for the more light receiving tip over the overshaded base.

The general scattering of the organisms discussed here over the plants was studied by the first author by scoring their numbers in at least eight squares projected on a leaf from tip to base and recording their mean number per square. This was done in three zones; zone X contained leaves situated close to the main stem of the *Sphagnum* plant and, therefore, the oldest on a branch. Zone Z comprised the outer, youngest leaves of a branch, zone Y the intermediate part of the branch. At least two leaves per zone were examined. The results are shown in *fig. 6*. A representation of the composition of these totals is given in *fig. 7* in which young, intermediate and old specimens were scored by assessing the iron-encrustation and the pore composition (A, B, and C respectively). This figure shows the same trend in all three zones: a high percentage of young, not encrusted individuals in the upper parts of the substrate and a steady decrease of these with increasing depth. Along with this decrease an increase of old and heavily encrusted individuals is manifest. These findings can be explained by assuming a steady growth of the *Sphagnum* plant and a preference of the organisms for colonizing the virgin, best illuminated upper parts of the substrate. The somewhat binomial distribution shown in *fig. 6* can be explained with the use of the above-mentioned findings, together with the assumption that the alga reproduces periodically. In these relatively short periods a sort of "birth explo-

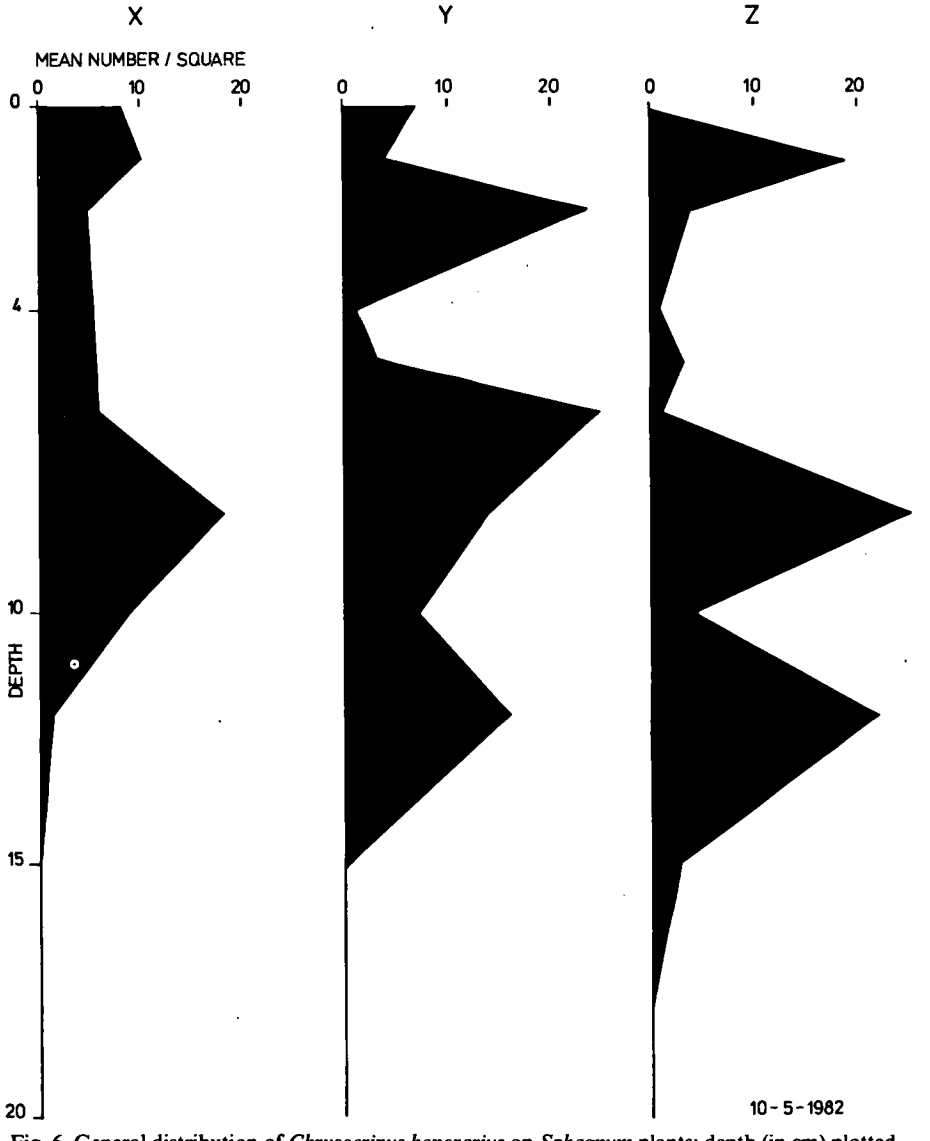

Fig. 6. General distribution of *Chrysocrinus honorarius* on *Sphagnum* plants; depth (in cm) plotted against mean number of epiphytes per square in three distinct zones (see text).

sion" takes place and the organisms start settling on the substrate in large numbers.

The second author studied in detail the numerical distribution in one plant in this case no distinction being made as regards the lorica type. Leaves from the zones X, Y and Z (*fig. 8*) were sampled and provided with incisions in order

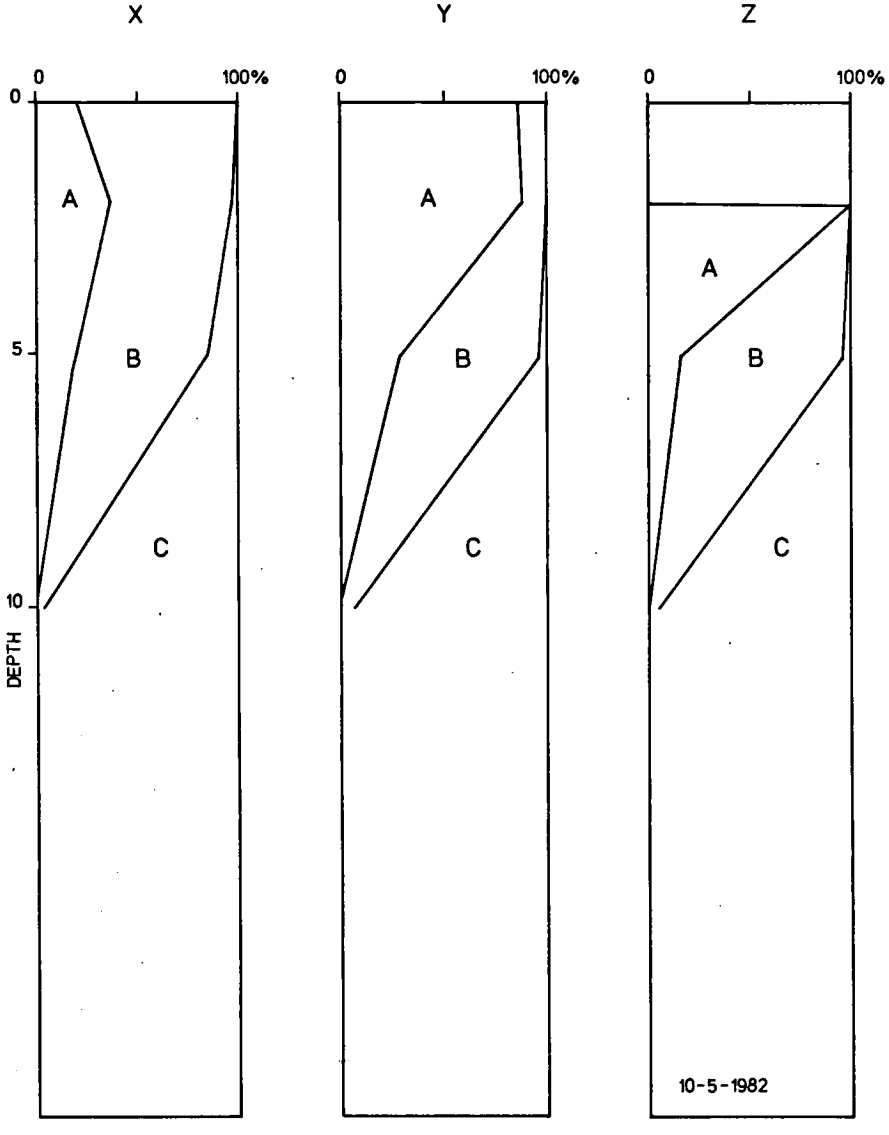

Fig. 7. Distribution of different stages of *Chrysocrinus honorarius* on *Sphagnum* plants; depth (in cm) is plotted against the percentage of occurence of young (A), intermediate (B) and old (C) algae. This figure was made by grouping all algae as encountered and shown in *fig. 6* into the three categories.

to make them lie as flat as possible. The numbers of specimens were scored in a series of fields of vision using an objective of × 40 magnification (area 70700 μm^2) positioned along the main axis from tip to base; this was done on both sides of the leaf successively. If half or more of the flat base of a specimen was

Table 1. Number of individuals of *Chrysocrinus honorarius* observed on leaves of a plant of *Sphagnum crassicladum* var *obesum*. L = number of leaf, F = number of field of vision (1 = tip), T = total of column, H + C = total of H(yalocyte) and C(hlorocyte) columns of a leaf. Further explanation the in text and the legend of *fig. 8*.

	L▶	1		2		3		4		5		6		7		8		9	
	F▼	H	C	H	C	H	C	H	C	H	C	H	C	H	C	H	C	H	C
ABAXIAL	1	0	0	0	0	2	13	0	0	5	38	0	7	5	59	5	72	10	44
	2	0	0	0	0	2	26	0	1	4	28	0	0	3	41	8	62	2	37
	3	0	0	0	0	0	6	0	0	1	25	0	0	4	64	7	51	0	28
	4	0	0	0	0	0	0	0	0	3	26	0	0	1	25	7	50	0	17
	5	0	0	0	0	0	0	0	0	0	1	0	0	4	17	3	52	2	9
	6					0	0	0	0	0	0			0	9	6	40	0	9
	7							0	0	0	0			0	9	3	34	0	0
	8							0	0	0	0			0	2	3	50	0	0
	9							0	0					0	1	2	27		
	10													0	0	0	4		
	11													0	0	0	0		
	12													0	0	0	0		
	T	0	0	0	0	4	45	0	1	13	118	0	7	17	227	44	442	14	144
	H + C	0		0		49		1		131		7		244		486		158	
ADAXIAL	1	0	0	0	0	0	0	0	0	0	0	0	1	0	4	0	0	0	3
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	4	0	5
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
	4	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0
	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	6					0	0	0	0	0	0	0	0	0	0	0	0	0	0
	7							0	0	0	0			0	0	0	0	0	0
	8							0	0					0	0	0	0	0	0
	9							0	0					0	1	0	0		
	10													0	0	0	0		
	11													0	0	0	0		
	12													0	0	0	0		
	T	0	0	0	0	0	0	0	0	0	0	0	1	0	7	1	6	0	8
	H + C	0		0		0		0		0		1		7		7		8	

attached to a chlorocyte, it was scored as chlorocyte dwelling, *mutatis mutandis* as hyalocyte dwelling. Discrepancies in the numbers of fields of vision is caused by the fact that it is impossible always to follow exactly the same track on both sides of the leaf; there can, moreover, be a difference in the folding and undulation in the alternative positions resulting in a somewhat larger or smaller area comprised in a field of vision. Although a near perfect flattening of the leaf under the cover glass is possible, this usually results in cracking the relatively fragile specimens. The scores are given in *table 1* and symbolized in *fig. 8*. The numbers scored were subjected to a goodness-of-fit-test, viz., the G-test advocated by SOKAL & ROHLF (1969).

The null hypothesis was equality of numbers:

10		11		12		13		14		15		16		17		18		19		20		21	
H	C	H	C	H	C	H	C	H	C	H	C	H	C	H	C	H	C	H	C	H	C	H	C
6	85	5	51	2	8	7	44	4	51	0	6	0	6	1	4	0	0	0	9	0	1	0	0
5	56	12	69	0	6	1	10	5	46	0	0	0	1	0	6	0	0	0	8	0	1	0	0
1	24	3	33	0	15	2	54	7	24	0	1	0	2	0	2	0	2	0	2	0	1	0	0
0	16	3	21	1	0	2	14	3	26	0	5	0	2	0	3	0	2	0	2	0	1	0	0
0	28	4	49	0	1	1	4	2	25	0	5	0	4	0	6	0	1	0	1	0	0	0	0
0	24	7	69	0	3	1	4	1	17	0	0	0	8	0	0	0	0	0	0	0	0	0	0
0	23	0	36	0	0	0	7	0	3	0	0	0	10	0	0	0	0	0	0	0	0		
0	23	5	58	0	1	1	6	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
0	14	0	26			0	5	0	0					0	0	0	1			0	0		
		0	11			0	2	0	0					0	0					0	0		
		0	3											0	0					0	0		
		0		0																0	0		
12	293	39	426	3	34	15	150	22	192	0	17	0	33	1	21	0	6	0	22	0	4	0	0
	305		465		37		165		214		17		33		22		6		22		4		0
3	24	0	3	0	0	1	4	3	15	0	1	0	6	0	0	0	0	0	1	0	0	0	0
1	26	0	4	0	0	0	1	0	5	0	0	0	6	0	0	0	0	0	0	0	0	0	0
1	17	0	0	0	0	0	5	0	5	0	0	0	3	0	0	0	0	0	0	0	0	0	0
0	1	0	3	0	0	1	3	0	2	0	0	0	4	0	0	0	0	0	0	0	0	0	0
0	7	0	4	0	0	0	0	0	1	0	0	0	3	0	0	0	0	0	0	0	0	0	0
1	6	0	2	0	0	0	2	0	0	0	0	1	7	0	0	0	0	0	0	0	0	0	0
0	6	0	3	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0		
0	5	0	2	0	0	0	1	0	0	0	0			0	0	0	0	0	0	0	0		
0	4	0	0	0	0	0	1	0	0					0	0	0	0			0	0		
		0	0			0	1	0	0					0	0	0	0			0	0		
		0	0											0	0					0	0		
		0	0											0	0					0	0		
6	96	0	21	0	0	2	19	3	28	0	1	1	29	0	0	0	0	0	2	0	0	0	0
	102		21		0		21		31		1		30		0		0		2		0		0

I on the adaxial and abaxial side of the leaf

II.1 on the apical and basal part on the abaxial side

II.2 ditto, on the adaxial side

(In case the number of fields of vision was odd the middle one of the series was left out.)

III.1 on the chlorocytes and hyalocytes on the abaxial side

III.2 ditto, on the adaxial side.

The results of the computations are presented in *table 2*. CS being higly significant in I confirms that over the whole plant the lower surface of the leaves is occupied by a greater number of specimens than the upper side; but significance of HET indicates that the pairs of scores on the leaves differ among themselves, i.e. the occupation is influenced by the position of the leaf on the plant.

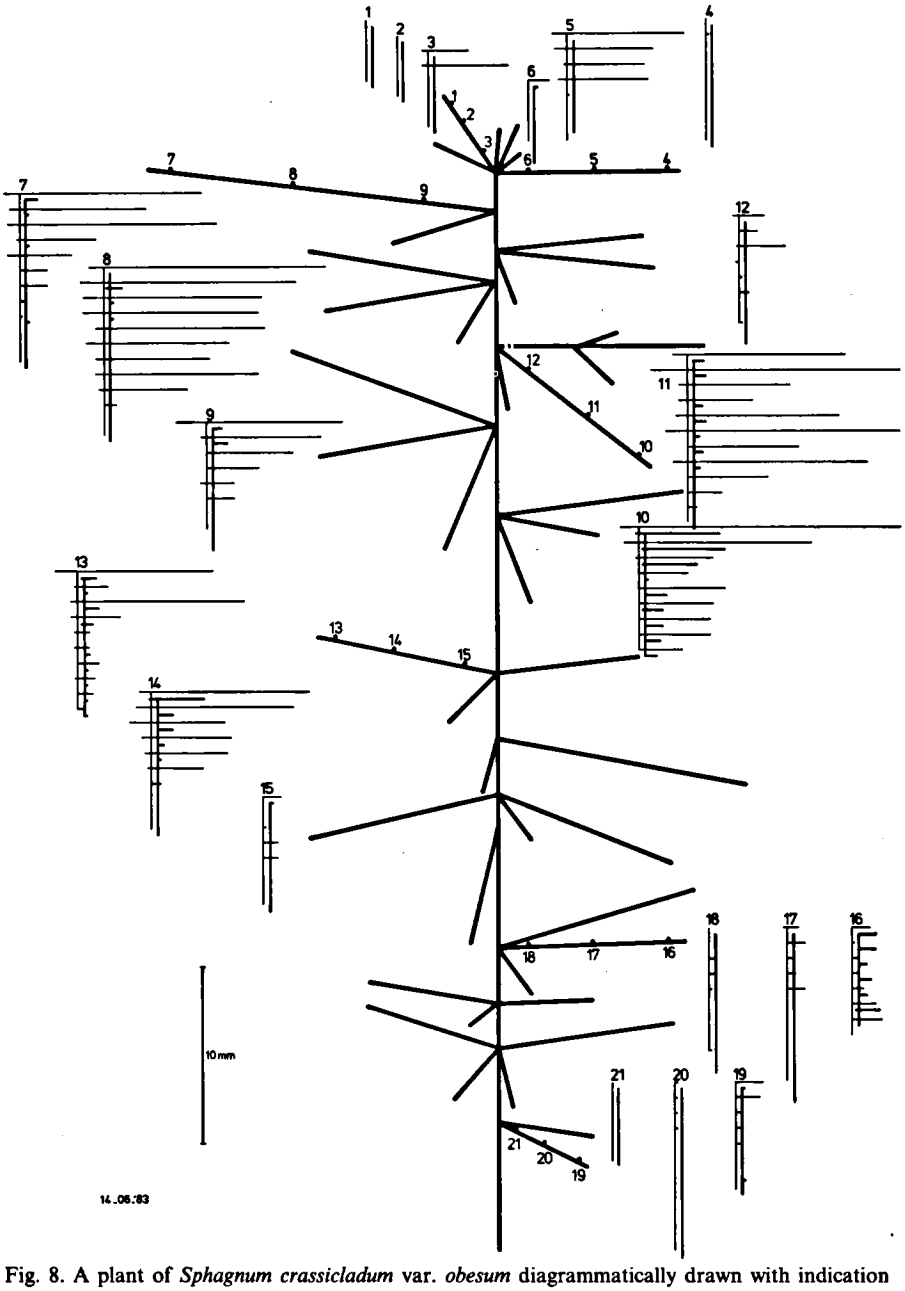

Fig. 8. A plant of *Sphagnum crassicladum* var. *obesum* diagrammatically drawn with indication (by numbers) of the leaves on which specimens of *Chrysocrinus honorarius* occupying hyalocytes (to the left of the vertical line) and chlorocytes (to the right of the vertical line) had been scored in a row of fields of vision on the main axis on both the abaxial (thin lines) and the adaxial (thick lines) surfaces.

Table 2. Number of observation pairs (N), value of G over the column sums (CS), the probability (prob CS), the heterogeneity of the individual observation pairs, viz., of the leaves (HET), and the probability (prob HET).

	I	II.1	II.2	III.1	III.2
N	18	18	11	18	11
CS	2041.492	921.553	81.136	1986.789	220.165
prob CS	$\ll 0.005$	$\ll 0.005$	$\ll 0.005$	$\ll 0.005$	$\ll 0.005$
HET	293.933	247.513	29.283	27.145	6.986
prob HET	$\ll 0.005$	$\ll 0.005$	< 0.005	> 0.05	> 0.05

The same holds for II.1 and II.2: the significance of CS justifies the conclusion that the apical parts are more densely occupied than the basal parts on either side of the leaf. A position effect is also indicated by the significance of the respective HET values, although it seems a little bit less strong at the inner side.

As regards III.1 and III.2 it can be concluded from the fact that the CS values are significant and the HET values are not that the chlorocytes are preferred as a substrate over the hyalocytes irrespective of the position of the leaf on the plant. The heterogeneity of the leaves may, as already pointed out, be explained satisfactorily by a difference in age on the one side and by the available quantities of light on the other (*fig. 8*). The greater availability of light, but possibly also the better contact with the surrounding water might be responsible for the denser occupation of the lower surface of the leaves. As can be extracted from *fig. 8*, also the uppermost and the lower leaves are more sparsely occupied which may be explained by the insufficient time available for colonization and the light becoming a limiting factor respectively; probably for similar reasons the apical (zone Z) and basal (zone X) parts of branches in the central part of the plant are relatively more sparsely colonized.

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