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Classe des Sciences Mathématiques et Naturelles. — Série B: Sciences Naturelles
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On the morphology and systematical value of the mycetozoon *Kleistobolus pusillus* Lippert

by

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O morfologji i systematycznej wartości śluzowca Kleistobolus pusillus Lippert. — On the morphology and systematical value of the mycetozoon Kleistobolus pusillus Lippert.

Mémoire

de M. *J. JAROCKI*,

présenté, dans la séance du 8 Novembre 1926, par M. K. Janicki m. c.

(Planche 20)

It is a source of great satisfaction to have found this remarkable mycetozoon in the Polish Tatra, as it is a rediscovery of the true *Kleistobolus pusillus* described by the late Christian Lippert in 1894. Since that date this rare species has not been found again till the present day. The extremely minute and scarcely visible sporangia, the dimension of which vary from 40 to 200 μ, have been probably overlooked by all specialists. The Tatrian specimens, which have been also authenticated by Miss Gulielma Lister, show in all essential respects the characteristic features of the forgotten form of Lippert.

I have collected the specimens in July 1926, in one locality only, at an altitude of nearly 1300 m. on the Krokiew-Mount (1378 m.) near Zakopane in the Tatra Mountains, Poland. The sporangia were found in some abundance, associated with *Comatricha nigra* Schroet., *C. laxa* Rostaf. and *Enerthenema papillatum* Rostaf., on a rotten ecorticated trunk of *Picea excelsa*. They are quite inconspicuous and almost too minute to be detected with facility, except by a well trained eye. It is worthy of notice that the Polish finding-place of this species is the first natural one, because Lippert found it in a chamber-culture.

With regard to the systematical position of the genus *Kleistobolus* we find in the literature entirely erroneous data, which may lead to false conclusions. In the second (1911, pag. 187—188) as well as the third edition (1925, pag. 182) of A. a. G. Lister's famous „Monograph of the Mycetozoa", this well distinct form is transferred by Miss G. Lister unfortunately to the genus *Licea* and given as a synonyme for *Licea minima* Fries. Prof. H. Schinz also follows Miss Lister in regarding this mycetozoon only as a synonyme for the same *Licea*[1]). Miss Lister writes that „Glycerine preparations of the type of *Kleistobolus pusillus* Lippert, kindly lent by Prof. v. Höhnel, show this to be a nearly typical example of the present species[2]). The rudimentary capillitium-threads described by Lippert are fungus hyphae traversing the substratum on which the sporangia are seated; the spores are faintly warted and measure 11 to 13 μ, not 7 to 9 μ, as stated by Lippert". We find nevertheless no mention in this Monograph that Lippert's original description and illustrations of *Kleistobolus*[3]) do not correspond to the features of true *Licea minima* Fries.

After an examination of the Tatrian specimens which were submitted to Miss Lister, she explains her mistake in a letter to myself by stating that in Jan. 1911 Prof. v. Höhnel, in response to her request to receive a mounting of Lippert's type of *Kleistobolus*, sent her under that name 2 mountings of small sporangia of *Licea minima*. These mountings led her astray.

On my part, I think, there was no good reason for rejecting the genus *Kleistobolus* without any restriction, especially as the description and drawings of Lippert are sufficient for the acceptation of his form even in spite of the eventual mistake on the part of v. Höhnel.

The results obtained by detailed examination of the mentioned gatherings from the Tatra fully confirm the expressed opinion, and not only make possible a more complete description of Lippert's species but allow to establish its constancy.

[1]) H. Schinz. Myxogasteres (Myxomycetes, Mycetozoa); in Rabenhorst's Kryptogamen-Flora von Deutschland, Oesterreich und der Schweiz. 2. Aufl. Leipzig, 1920.

[2]) Viz. *Licea minima* Fries.

[3]) Ch. Lippert. Ueber zwei neue Myxomyceten; in Verhandlungen der k. k. zoologisch-botanischen Gesellschaft in Wien. XLIV Bd. Jahrg. 1894. pag. 70—72. tab. III—IV.

Kleistobolus pusillus Lippert.

Plasmodium pale blackish-brown (according to Lippert). Sporangia sessile, operculate, very minute, 0·04—0·2 mm., solitary scattered on the substratum or in small groups; tympan-or kettle-shaped, rarely subglobose, normally circular, sometimes ellipsoidal or lightly elongated in contour. Peridium simple, membranous, pale brownish, more or less opaque from dark brown deposits of dis-charged organic matter, except in the narrow upper margin, which is usually almost free of these deposits and terminated by a continuous row of numerous peg-like, hyalin colourless warts, about 1—2 μ in diam., rarely glossy from scanty inclusions. Sporangia opening by an usually very distinctly developed membranous, thin, pellucid and colourless circular operculum, shining with iridescent reflections. It is usually plate-shaped with lightly convex centre and a distinct, more or less broad, circular border, which often forms an angle with the boundary of the upper margin of the sporangium, occasionally simply convex, flattened or concave. It is distinctly papillose: on the periphery of the inner surface from included numerous prominent, bead-like hyalin refracting warts or globulae, 1 to 2 μ. in diam., situated in some intervals from each other, in the remaining, more central parts dotted with scarcely visible scattered papillae. The boundary between the larger marginal and the minute centrally distributed warts is more or less distinct. The lid is charged besides with short, irregularly finger-shaped, blunt at the tips, tubular hollow processes, about 10—15 μ. long, 2—4 μ. broad, attached inwards to the various parts of the lid. These processes are indefinite in number and in arrangement. Spores almost smooth or minutely verruculose from more or less defined, thickened areas of the exine separated by thinner bands, pale lilac-brown or pinkish in mass, pale brownish or nearly colourless when highly magnified, 9 to 11 μ. in diam.

Habitat. 1. In a chamber-culture found associated with *Colloderma oculatum* (Lipp.) G. Lister and *Cribraria* sp., on moist stick of fir-wood *(Abies)* from Hirschaualpe (Salzkammergut) near Hallstatt in Upper Austria. (Ch. Lippert IV—1893). — 2. On rotten wood of *Picea excelsa;* Krokiew-Mount near Zakopane in the Tatra, Poland (leg. J. Jarocki VII—1926).

As to the systematical relationship of *Kleistobolus*, Lippert himself considers the presence of the „rudimental capillitium“ as sufficient for placing it near *Perichaena*, but it is difficult to agree with his opinion about the capillitial character of the tubular outgrowths situated on the lid. On the other hand, he expresses his opinion founded on the erroneous in reality not existing „auffallende Eigentümlichkeiten“, that *Kleistobolus* is to be inserted as the type of a new „fünfte Familie unter die *Coelonemeen*... und zwar zwischen die *Perichaenaceen* und *Liceaceen* Zopf's“ (loc. cit. pag. 72). The mentioned theoretical considerations based upon his inaccurate studies are of no value. In point of the fact, the correct position of this genus lies doubtlessly in the family of *Liceidae*.

It appears to be allied in some respects on one side to *Hymenobolina* and on the other to *Orcadella*. *Kleistobolus* differs from both not only externally in the shape of the sporangia, but especially in the very peculiar structure of the operculum, the dentate margin of the peridium and the different spores. The differences between *Kleistobolus* and the two other similar monotypic genera *Hymenobolina* and *Orcadella* are tabulated below. A comparison of the features of *Kleistobolus* with those of the already mentioned forms allows us to state that the morphological values of the discussed genus are really important and quite sufficient to separate it from the other known genera of *Liceidae* (vide plate pag. 853).

The sporangia of *Kleistobolus pusillus* resemble superficially at first sight *Hymenobolina,* but differ in the more distinct, plate-like sporangial lid which is usually brilliantly iridescent and also when magnified shows papillose appearance. This appearance is not caused by the impression of the spores, but by the numerous spherical warts, imbedded in the lid. After a detailed examination the present species cannot be confused with *Hymenobolina*, because of certain very characteristic pecularities, such as: the remarkable disposition of the above mentioned warts, the tubular processes of the lid, the dentate margin of the peridium and also the paler and smaller spores with in definite area of dehiscence.

The specimens are normally regular tympan-like, but in the large gatherings sporangia are found which show some variations in the shape viz. having an ellipsoidal or elongated and not orbicular outline. The form of the operculum is in such cases adapted

	Kleistobolus Lippert.	**Hymenobolina** Zukal.	**Orcadella** Wingate.
The shape of the sporangia.	Sporangia sessile, tympan- or kettle-shaped rarely subglobose, opening by a well-defined plate-like lid.	Sporangia sessile, subglobose or forming short plasmodiocarps, opening irregularly or by a lid.	Sporangia stalked, urn-shaped or subglobose, opening by a well-defined lid or in var. *sessile* along one or more ridges.
The structure of the peridium.	Peridium membranous, no papillose; the margin of the sporangium-wall serrated with continuous row of globular warts, about 1—2 μ. diam.	Peridium membranous very subtile papillose; the margin of the sporangium-wall smooth.	Peridium cartilaginous; the margin of the sporangium-wall smooth.
The structure of the operculum.	Operculum colourless, usually plate-like with more convex central part and a distinct border, which usually forms an angle with the dentate margin of the sporangium, rarely single convex, flattened or concave, charged on the periphery of the inner surface with prominent bead-like hyalin warts, 1 to 2 μ. diam., dotted with minute scattered papillae in the centre and provided with short tubular processes, which project inwards from the lid.	Operculum smooth or areolated with prominent ridges, flattened or lightly convex, very minutely papillose from densely accumulated on the inner surface subtile warts.	Operculum dull-yellow, rough from prominent irregular ridges, flattened, convex or dome-shaped, charged on the inner surface with very minute papillae and invested with granular deposits of the discharged matter; absent in var. *sessile*.
The structure and size of the spores.	Spores globose almost smooth or minutely verruculose from thickened areas of the spore-wall, pale lilac-brown in mass, pale brownish or nearly colourless when magnified; 9—11 μ.	Spores subglobose, the spore-wall with well-defined thin, colourless area of dehiscence, shining chestnut-brown in mass, brown when magnified; 11—16 μ.	Spores globose smooth, yellowish or pink in mass, almost colourless when magnified, or lilac-pink in var. *sessile*; 8—11 μ.

to the modified shape of the sporangia. Sometimes the sporangia are abnormally developed, irregular or confluent, occasionally they have no lids, but the peridium is dark all over with discharged matter. We rarely found open sporangia with the lid bent aside or with an already fallen lid. It is possible also to separate the lid from the lower margins of the sporangium by light pressure with a thin needle. The dark masses of the discharged amorphous substance invest the peridium more or less abundantly in the majority of sporangia but sometimes they are almost absent. In such cases the sporangium-wall is glossy or iridescent.

With regard to the operculum of *Kleistobolus,* it is very strange that Lippert gives so erroneous an explanation about its relation to the sporangium-wall and remarks here an „abweichende Eigentümlichkeit" from all other Mycetozoa, describing the lid as lying on the bottom surface of the sporangium „Der Deckel" wrote Lippert „bildet die Basis des Sporangiums und letzteres löst sich vom ersteren los". In reality, he must have got all the examined sporangia in his preparations overturned in a drop of water which is not surprising with so minute an object.

The structural characteristics of the operculum show many differences with those of *Hymenobolina* and *Orcadella.* The lid of *Hymenobolina parasitica* is so minutely papillose, that this feature can be observed only when highly magnified. According to H. Zukal, the peridium of *Hymenobolina* is „subtilissime punctatum" and shows „unter dem Immersionssysteme eine dichte aber sehr feine Punktierung, welche mir von äusserst zarten, nach aussen vorspringenden, soliden Wärzchen herzukommen scheint"[1]). E. Jahn has described his *Licea singularis* (the species, which is placed by Miss Lister as a synonyme for *Hymenobolina parasitica* Zukal) as having so minute papillae that these „erst bei Anwendung einer $^1/_{12}$ Öl-Immersion hervortreten. Sie sitzen (Fig. 8) oft nahe zusammen und bilden geschlängelte Reihen, die in bestimmtem Abstand von einander bleiben und ein zierliches Muster bilden"[2]). The same may be induced from the illustrations of *Hymenobolina* in Lister's

[1]) H. Zukal. Ueber zwei neue Myxomyceten. Österreichisch. Botan. Zeitschr. XLIII Jahrg. 1893. l. cit. p. 133.

[2]) E. Jahn. Myxomycetenstudien. 9. Bemerkungen über einige seltene oder neue Arten; in Bericht. d. Deutsch. Bot. Ges. Jahrg. 1918, Bd. XXXVI, 1919 l. cit. p 666.

Monograph. With regard to the subtile papillae of the lid of *Hymenobolina*, it is to be remarked that they are in all essential respects identical with the mentioned sculptures of its sporangium-wall. On my part, I have had opportunity to confirm on specimens of English provenience that the discussed features exactly agree with the preceding description. As for *Orcadella operculata* Wingate, which species I have had also under examination, it has also a very minutely papillose lid, invested moreover with scattered deposits of dark discharged matter, as is well illustrated by Miss Lister. As to the peridium of *Kleistobolus* it is not at all papillose, as stated already by Lippert who writes: „nur die Innenseite des Deckels mit runden glashellen Körperchen übersäet ist, während die übrige Innenwand des Sporangiums mit Ausnahme des geperlten Randes keine Spur ähnlicher Körnchen besitzt". Nevertheless Lippert erroneously describes the peridium as „doppelt, von einer inneren zarten, farblosen und einer äusseren dicken, dunkel gefärbten Hautschichte gebildet". As a matter of fact, it consists of the only one pellucid, lightly pale-brownish membranous layer, covered more or less abundantly with dark deposits of the discharged substance which occasionally may be almost absent. The discussed form, as all species of *Liceidae*, does not contain carbonate of calcium.

The capillitium is completely absent. The peculiar tubular processes, „kurze Röhrchen" of Lippert, which are attached to the inside of the lid are, as I suppose, probably nothing more than very modified bead-like warts, scattered on the lid. For the elucidation of the rôle played by these outgrowths in the sporangium, further investigations are needed. The application of the term „rudimental capillitium" to the mentioned outgrowths of *Kleistobolus*, as Lippert has done, appears inappropriate and precocious.

It is known that the capillitium does not exist in any of the species of *Liceidae*. On the other hand, Dr. M. Brandza lately has described in *Licea minima* the trace of a „rudimental capillitium". He writes: „La constance avec laquelle nous avons constaté dans presque tous les sporanges de *Licea minima* Fries quelques filaments jaunâtres qui ont tous les caractères d'un capillitium rudimentaire, nous fait croire que les observations de Lippert à ce sujet ne sont pas denuées de tout fondement. Il est peu probable que les hyphes des champignons réussissent à pénétrer régulièrement

dans chaque sporange“ [1]). I quite agree with this point of view of Dr. Brandza. I myself had the opportunity of confirming the presence of scanty, short processes situated occasionally on the inner surface of the peridial lobes of *Licea minima*. Miss Lister gives also, in a letter to myself, a pencil sketch of such little processes in the same *Licea*.

Very similar relations have been described by Miss Lister in *Orcadella operculata* Wingate var. *sessile* G. List. Among some sporangia which she has examined „in one sporangium two slender spikes enclosing dark refuse matter project inwards from the walls, while a longer spike forms a bar, varying from 5 to 10 μ. diam., crossing the sporangium and connecting the opposite walls. Possibly this is not a normal development; the spores are, however, perfectly formed“ [2]). This is one more instance where a representative of the *Liceidae* shows a structure having considerable resemblance with a true capillitium [3]).

H. Zukal has also described in *Hymenobolina parasitica* a „rudimental capillitium“ without calcium „in singularibus locis cum peridio coalescens circa 0.5 μ. latum, solidum apparens, non multum racemosum, laevigatum, hyalinum, interdum prorsus desideratum“ (vide Zukal, l. cit. pag. 73, etiam tab. V, fig. 3), which „Auf jeden Fall kann... keinen nennenswerten Einfluß auf die Ausstreuung der Sporen nehmen“ (ibid. l. cit. pag. 134). Miss Lister, however, has remarked that „there is no trace of capillitium in the sporangia we have examined, and it is probable that the threads Zukal described as capillitium were the hyphae of some minute mould“. This opinion with regard to the drawing of Zukal seems to be very probable. I myself had no opportunity of verifying Zukal's observations, because I had only scanty material of the *Hymenobolina*.

[1]) M. Brandza. Nouvelle note sur quelques myxomycètes récoltées en Roumanie. Annales scient. de l'Université de Jassy, T. XI; 1923. loc. cit. pag. 411.

[2]) A. a. G. Lister. A Monograph of the Mycetozoa. 3. edit., 1925. loc. cit. pag. 186.

[3]) Dr. Gusztáv Moesz has recently published („Myxomycetes“ in: Folia cryptogamica, vol. I, num. 3, 1925, Szeged; pag. 159—160, 193.) a new species-*Licea hungarica* with scanty capillitium from Süköd (Comitat Pest), Hungary. Through the courtesy of Dr. Moesz (Magyar Nemzeti Múzeum, Budapest) I was able to study cotype-specimens (Süköds, VII-1919; leg. F. Greinich) of this mycetozoon, but the presence of rudimental capillitium has not been observed in the sporangia which I have examined.

The absence of a capillitium in *Liceidae* is usually considered as an undoubtedly primitive feature. On the other hand it is difficult to say, on the basis of so scanty observations, something about the morphological and phylogenetical value of the mentioned „rudiments" or capillitium-like structures without falling into error. I think also, that further and more precise investigation of the numerous sporangia of certain kinds of *Liceidae* as well as cytological researches into development of the sporangia, will definitely clear up this question as to an analogy or even perhaps homology of the discussed structures. I suppose, however, that the absence of a capillitium in *Liceidae* might be of a secondary and not a primary nature. At any rate, in some Mycetozoa which usually have a more or less abundant capillitium, it may be completely absent. For instance we found no capillitium or very scantily in *Perichaena quadrata* Macbr. and in *Per. corticalis* Rostaf. var *liceoides* List. *Oligonema flavidum* Peck was found occasionally without it. Mr. A. Pouchet has recently found no trace of the capillitium in six successive gatherings of his *Olig. nitens* Rostaf. var. *anomalum* Pouchet[1]).

The row of marginal warts on the lower border of the sporangium-wall of the *Kleistobolus*, which are absent in *Hymenobolina* and also in *Orcadella,* may be compared with the papillae situated along the lineae of dehiscence on the peridial lobes of certain species of *Licea.* It is very probable, as Lippert supposes, that the marginal warts of the lid and of the border of the peridial-walls serve for shutting the sporangia, cooperating with each other as a closing mechanism.

It may be here added that the spores of *Kleistobolus pusillus* resemble in size those of *Orcadella*, but differ from them in mass by their pale lilac-brown colour. The var. *sessile* of *Orcadella operculata* differs from our species in the irregular manner of dehiscence and in the spores.

[1]) A. Pouchet. Contribution à l'étude des Myxomycètes du Département du Rhône. Annales Soc. Linnéenne de Lyon. Ann. 1925, nouv. sér. t. LXXII, pag. cit. 60.

Table of the genera of Liceidae.

A. Sporangia sessile, subglobose or forming plasmodiocarps, dehiscing irregularly or by regular lobes . . . *Licea.*

B. Sporangia operculate.

1. Sporangia sessile, lid very minutely and densely papillose . . . . . *Hymenobolina.*
2. Sporangia sessile, lid charged with scattered globular warts, 1—2 μ. diam., and with short tubular processes . . . . . *Kleistobolus.*
3. Sporangia stalked, very rarely sessile, opening by a minutely papillose lid, or by one or more ridges . . . . . *Orcadella.*

I am deeply indebted to Miss Gulielma Lister, F. L. S., London, for her invaluable assistance and kindness in sending me the specimens of *Hymenobolina parasitica* and *Orcadella operculata*, which species have not yet been found in Poland.

Institutum Zoologicum Universitatis Varsoviensis. Warsaw, September 1926.

Explanation of Plate 20.

1. *Kleistobolus pusillus* Lippert, a group of typical sporangia, × 125.
2. Serrate margin of the peridium with a row of globular warts, × 500.
3. Operculum showing a characteristic structure and tubular processes on the inside, × 500.
4. Spores, × 660.

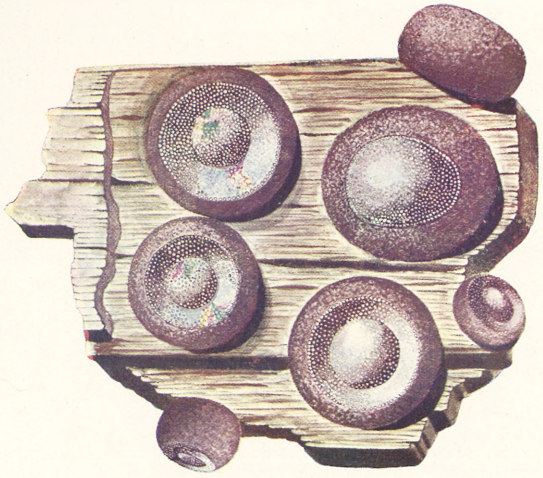

Fig. 1.

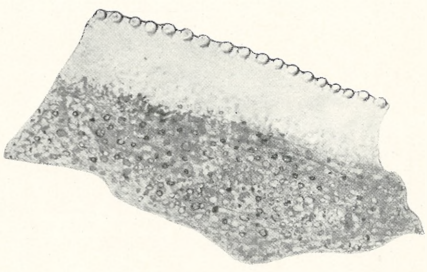

Fig. 2.

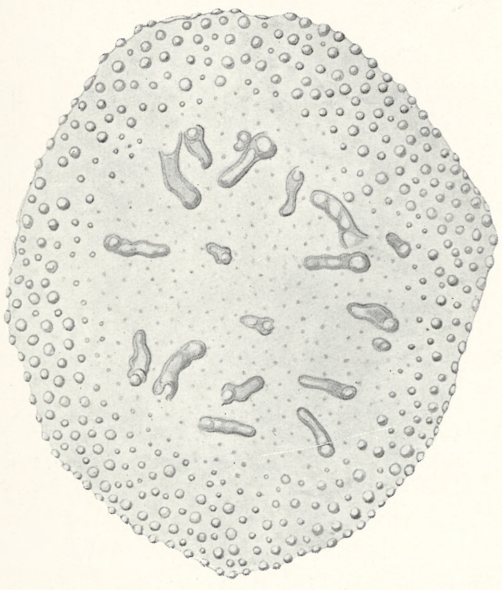

Fig. 3.

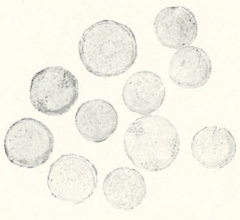

Fig. 4.

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