ACTA
MYCOLOGICA
vol. VII. 2—1971

A new species of *Pythiogeton* showing *Achlya*-like diplanetism

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In November 1967 I collected from a large old bed of the Vistula near the Miedzeszyn dam in Warsaw a 2-litre sample of water and of the bottom sediment. The sample was subjected to long term observation in the laboratory for the purpose of finding saprophytic aquatic fungi and those parasitizing on algae. The sample was kept for one month at 10—13°C under natural daylight and additional illumination with a mercury lamp. The nutrient substances were supplemented by adding every week several drops of Bayer "Wuchsal" nutrient medium. The material was microscopically inspected at first once weekly, and in the end period of the observation, every day.

The motile *Chlamydomonadaceae* monads at first present in the natural material subsisted in the laboratory conditions for about 3 weeks, multiplying rather intensively, they exhibited, however, contrary to expectations, no traces of infestation with parasitic fungi. In the second week of observation I threw into the dish as bait several fruits of *Sorbus intermedia* Pers. with the stalks and short shoots without taking off the dried up dead and black fruits. After a few days on the surface of the stalks and dead fruits I noticed a delicate web-like blakish deposit which soon spread to the whole surface, without, however, passing to the epidermis of healthy fruits. This web consisted of a loose weft of thin isodiametric, poorly branched, straight running hyphae. The wall of the hyphae was thin, smooth, blackish, greyish at the tips. At regular intervals short lateral processes grew from the hyphae bearing spherical zoosporangia and gametangia on their tips (Fig. 23).

After following the development of the fungus and observation of its sporogenesis, I decided that it is a member of a so far unknown species of the genus *Pythiogeton* Minden which I describe below as *Pythiogeton nigrescens* sp.nov.

Pythiogeton nigrescens sp. n.

Mycelium nonnulis ramis instructum, hyphis 2,3—4,6 μm *crassis, cum processibus brevibus perpendicularibus, longis* 120—300 μm et 0,7—3,0 μm *crassis, cum sporangiis vel gametangiis terminalibus, membrana levi, tenui, translucente, senio nigrescenti. Zoosporangia terminalia, sphaerica*

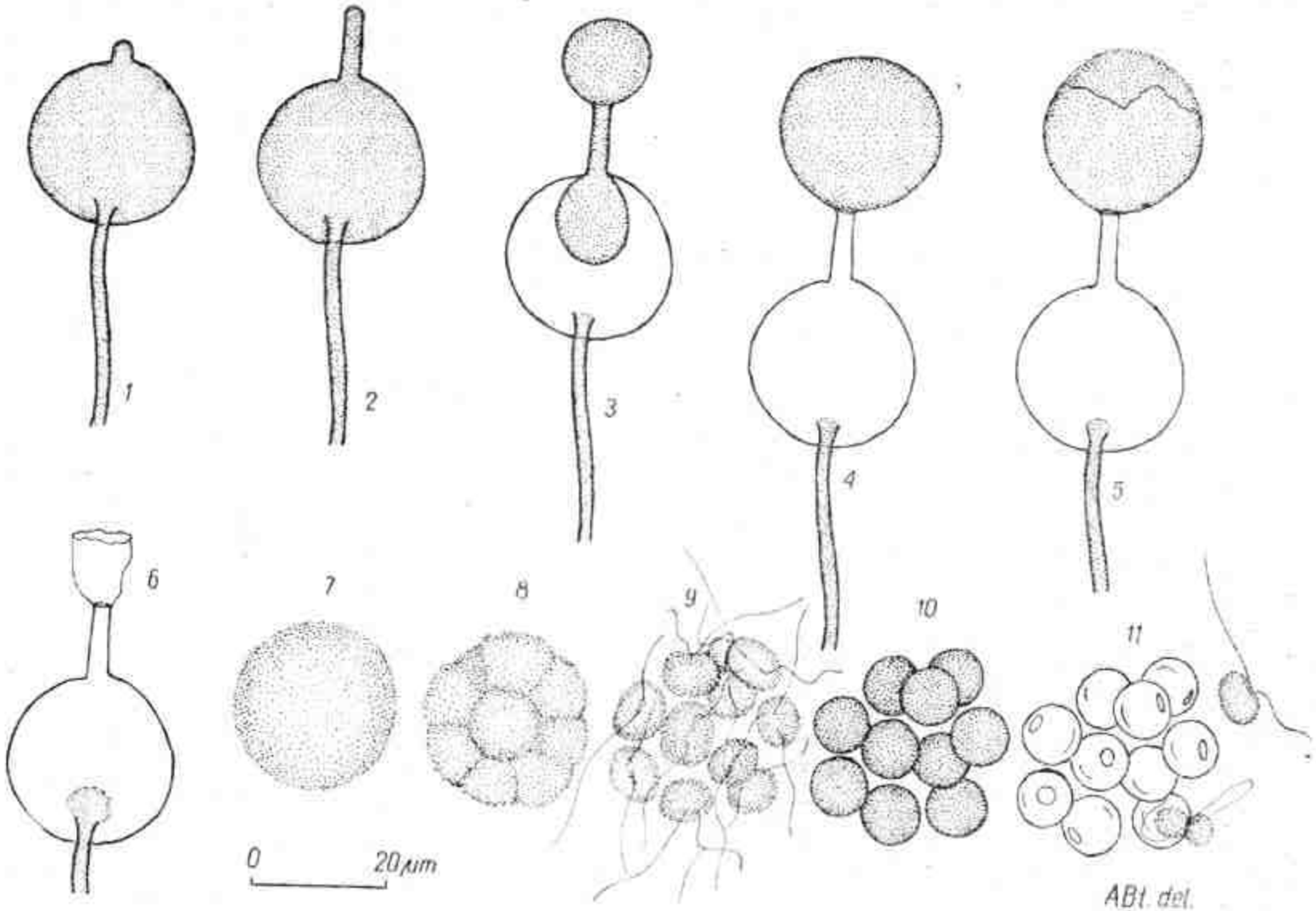
vel rarius late ovata vel late eliptica, 23,5—40,5 μm *in diametro, miedocriter circa* 33,0 μm, *proliferentia, tubulis evacuatoriis singularibus apicalibus vel lateralibus, longis* 7,5—20 μm, *crassis* 2,0—5,0 μm, *ornata. Zoosporae primariae late reniforme*, 8,8—14,2 μm *longae, saepius* 11,7 μm *longae, cum flagellis duobus inaequalibus ex massa sphaerica protoplasmatica nuda extra sporangium et vesiculum formatis, maturae inactivae, in situ incystentes, in sphaericas cystosporas primarias transmutantur, in diametro* 8,0—10,0 μm. *Zoosporae secundariae primariis similes sed activae natantes. Cystosporae secundariae primariis similes sed singulares. Oogonia apicalia, sphaerica* 25,0—35,0 μm *in diametro, cum uno oosporo plerotico, membrana lucida*, 6,8—8,5 μm *crassa. Antheridia diclina, apicalia in truncis, bursiformia vel subsphaerica*, 9,5—14,3 μm *in diametro.*

Habitat in detritu aquae immersus prope fluvium Vistulum; Varsovia in Polonia.

Typus: figurae 1—23.

The mycelium of *Pythiogeton nigrescens* forms in water culture delicate web-like wefts and consists of hyphae 2.3—4.6 μm thick exhibiting grey-black walls and a linear course. The thickness of the hyphae is more or less equal on all their length. The contents of the hyphae are fine-grained rather homogeneous and differ markedly from the protoplasm filling usually the hyphae of other members of aquatic *Pythiaceae* — they are not hyaline and do not contain strongly refractive elements. If we disregard the short lateral processes mentioned above, the hyphae of *P. nigricans* may be considered as nonbranched or very seldom branched. The not numerous long side branches depart from the axial hypha at an acute angle. The short lateral processes form on the hyphae at several hundred-micron intervals, they are somewhat thinner than the hyphae (usually 2.2—3.0 μm thick) and reach a length of 300 μm. Soon after their appearance the processes begin to swell at the tip and transform within several hours to stalked zoosporangia or gametangia.

Figs. 1—11. Zoosporogenesis in *Phythiogeton nigrescens*. Mature sporangium with incipient evacuation tube *(1)* nad fully developed evacuation tube *(2)*; outflow of plasmatic contents from sporangium into spherical visicle at its outlet *(3—4)*; beginning of release of plasmatic mass from vesicle which has burst at top and shrivels, slipping down to outlet of sporangium *(5)*; empty sporangium with remnants of vesicle at outlet and primordium of new sporangium proliferating into the old one *(6)*; naked plasmatic mass freely floating in water *(7)*; formation of cleavage furrows *(8)*; primary zoospores *(9)*; primary cystospores *(10)*; emergence of secondary zoospores and empty cystospores; pores are visible in the cystospore walls *(11)*. (Time-lapse drawings of the same sporangium made with the aid of camera lucida).



Figs. 1—11

The zoosporangia of *P. nigrescens* of almost spherical shape (less frequently ovoid or oval) are 23.5—33.0 μm in diameter. The are filled with fine-grained, sometimes slightly foamy plasma. When the zoosporangia reach their final size, a cylindrical evacuation tube (Figs. 1, 2) grows out from the side, wider than the supporting hypha. I did not succeed in finding a septum separating the developing sporangium from the supporting hypha. When the evacuation tube reaches a sufficient length the development of the sporangium is inhibited for long hours. I succeeded in following its development only once (Figs. 1—11). The particular phases of the process could be observed, however, repeatedly on various sporangia. In the case here described (Figs. 1—11) the period of rest of the mature sporangium lasted more than 8 hr, in the late evening hours the tip of the tube burst or dissolved unnoticeably, and the contents of the sporangium flowed out within more or less 30 sec, forming a ball attached to the tip of the evacuation tube. In the fungus here described I did not notice inside the evacuation tube of ripe sporangia any hyaline plugs like those frequently described in other members of *Pythiaceae*, e.g. in *Pythiogeton autossytum* Dreschler (Dreschler 1932, Batko and Żebrowska, in letter). The hyaline plug serves probably in the subsequent phase as material for the formation of a thin vesicle surrounding the plasma which flows out of the sporangium. This vesicle in the fungus described is extremely thin-walled, almost unnoticeable.

After a lapse of several minutes a noticeable change occurs in the appearance of the plasmatic ball: the thin-walled vesicle encapsulating it bursts at the tip, shrivels and slips off the ball exposing the bare plasma (Fig. 5). This ball is now detached and floats passively away from the outlet of the sporangium (Figs. 6 and 7). The shrivelled remnants of the vesicle remain attached like a short sleeve to the tip of the evacuation tube and are better visible now (Figs. 6, 18, 19). The way the plasma is released from the sporangium in the fungus described is distinctly different than in the remaining species of the genus *Pythiogeton* in which the plasma is rather vigorously ejected from the sporangium and remains in the water in the shape of an elongated jet coated with a vesicle. After a short period of rest it is ejected from the vesicle with such power that it often disappears from the field of vision of the microscope (Dreschler l.c.).

Zoospore cleavage in *P. nigrescens* starts several minutes after release of the cytoplasm from the vesicle. There first appear gradually deepening cleavage furrows dividing the ball into a dozen or so parts (Fig. 8). The formation of flagella begins somewhat later and occurs simultaneously with the just described process, so that the zoospores finally separated

from one another have already fully developed flagella (Fig. 9). About 8—12 such zoospores are formed, they are kidney shaped and measure 13.8—14.2 μm in length (average ca. 11,7 μm) and 6.3—9.1 μm in width (average ca. 8.2 μm). The ventral side of the zoospores is distinctly flattened. Through its middle runs a rather short well visible wide groove from which two opposed flagella of unequal length (16—27 and 34—40 μm) arise. The fully developed zoospores wave their flagella rather slowly without swimming away from their site. They only change slightly several times their position in the group so that their arrangement is somewhat loosened (Fig. 9). They lose their flagella after several minutes (probably they are drawn in, not rejected), become rounded and undergo encystation. Thin-walled spherical cystospores 8.0—10.0 μm in diameter remain in a state of rest for 3—5 hr, and then from each there emerges through an even-edged pore a secondary zoospore. The latter do not differ noticeably from primary ones either by their dimensions or shape, but in contrast to them they are very motile and immediately swim away. The release of the secondary zoospores from the cystospores constituting the given agglomeration occurs practically at the same time, more or less within half an hour. Sometimes part of the cystospores releases their zoospores with a delay of several hours or does not release them at all. The period of mobility of the secondary zoospores in the material observed exceeded 5 hr. Encystation was not noticed. Secondary cystospores were found in the material only several times, and their germination was not observed, therefore it was not possible to establish the number of phases of their mobility. On the other hand soruses of primary cystospores could be seen many a time, and I believe that the diplanetism and functional regression of the primary zoospores here described are obligatory traits of *P. nigrescens*, at least in water cultures of the fungus.

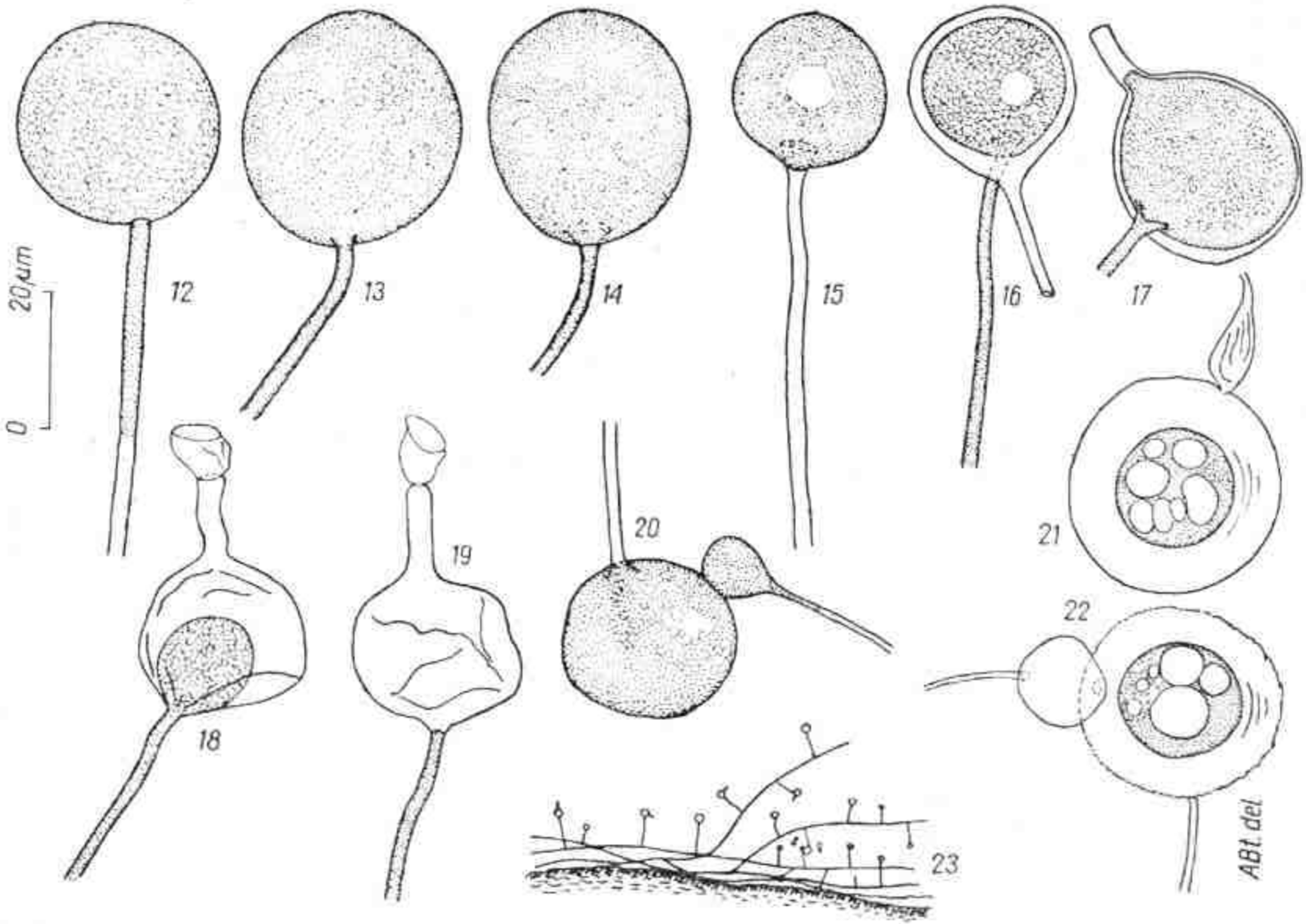
Immediately after ejection of the plasma, a process of internal proliferation could be usually observed (Figs. 5, 6, 18) in the empty sporangia of *P. nigricans*. The zoosporangia arising in this way filled the old sporangia and their evacuation tubes generally ran out through those of the old sporangia. Cases of perforation of the wall of the old sporangium by the evacuation tube of the new one were very rare, *P. nigricans* differing in this respect from *P. utriforme*, *P. autossytum* and others (cf. Dreschler l.c., Sparrow 1960).

In the end period of the original water subculture of *P. nigrescens*, relatively few oogonia and antheridia began to form. The young oogonia resemble at first young zoosporangia, but their contents are somewhat more coarse-grained and they are frequently vacuolized. The antheridia are smaller, pear-shaped, diclinic, they grow from other hyphae than the oogonia and grow towards them on straight or slightly bent stalks

(Figs. 20 and 23 ♀, ♂). Upon contact with the oogonium, the antheridium adheres to it at any site by a rather large surface area. I did not find in *P. nigrescens* the twisting of the oogonium and antheridium stalks round each ather as described in numerous species of *Pythiogeton*. Neither did I notice the formation of fertilization tubes: during the flow of the antheridium contents into the oogonium, no noticeable changes occurred *in vivo* in the content of the latter.

Soon after fertilization, the walls of the empty antheridium usually wrinkle slightly (Fig. 21), they may, however, preserve sometimes a smooth contour (Fig. 22). The oospore wall greatly thickens (up to 6.8—8.5 μm). In the content of the maturing oospore also distinct changes occur, several to a dozen or so lipid droplets randomly dispersed appear in it. The wall of the ripe oospore has three distinct layers: the episporium is very thin and frequently desquamated, the very thick exosporium is hyaline and layered; on the inner side it is lined with the thin plasmatic endosporium. On the surface of mature oospores there generally remain remnants of the antheridium and supporting hypha. Germination of the oospores was not observed. The course of zoosporogenesis and the unusual thickness of the zoospore wall are evidence favouring the assignment of the fungus here described to the genus *Pythiogeton* Minden. It differs, however distinctly from the six species listed in Sparrow's monograph (1960) by the spherical shape of the plasmatic vesicle and of the naked plasmatic ball from which the zoospores are formed. This fact would seem to be due to the low energy of plasma flow from the zoosporangium in this fungus. The scond significant difference is the functional regression of the primary zoospores owing to which *P. nigrescens* exhibits *Achlya*-like diplanetism. The diagnostic differences between *P. nigrescens* and the previously known *Pythiogeton* species are given in the form of Sparrow's key (Key to the species of *Pythiogeton*, Sparrow, 1960, p. 1044) suitably modified. The newly detected fact of sexual reproduction of *P. autossytum* Dreschler is included in this key.

Figs. 12—23. Morphology of *Pythiogeton nigrescens*. Zoosporangia of various shapes and dimensions *(12—15)*; internally proliferating *(16—18)* and empty zoosporangia *(19)*; oogonium and antheridium in phase preceding fertilization *(20)*; oogonia with mature thick-walled oospores *(21—22)*, empty antheridia are attached to both oogonia, and to the lower one remains of the supporting hypha; general habitus of a fragment of the mycelium on the surface of the stalk of a fruit of *Sorbus intermedia*, zoosporangia and gametangia are visible *(23)*. (Drawn with camera lucida, Fig. 23 free-hand drawing).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Key to the species of *Pythiogeton*

Sporangia bursiform or irregularly saccate

Sporangia for the most part terminal

Sporangia predominantly broadly bursiform, the discharge tube nearly parallel with the axis of the attendant hypha; sporangiferous hyphae sparingly branched; oogonia spherical

P. utriforme Minden

Sporangia predominantly narrowly bursiform, the discharge tube approximately at right angles to the axis of the attendant hypha; sporangiferous hyphae richly branched; sex organs unknown

P. ramosum Minden

Sporangia for the most part intercalary

Sporangia (four) averaging 154 μm long by 60.5 μm in diameter; oogonia polygonal

P. transversum Minden

Sporangia (one hundred) averaging 96 μm long by 42 μm in greatest diameter; oogonia spherical

P. autossytum Drechsler

Sporangia spherical or ovoid

Vesicle and nacked plasmatic mass oblong-ovoid or longer, primary zoospores actively swimming away

Sporangia 40—56 by 30—40 μm; discharge tube 39—70 μm long

P. uniforme Lund

Sporangia 20—34 by 18—28 μm; discharge tube 7.5—20 μm long

P. dichotomum Tokunaga

Vesicle and nacked plasmatic mass spherical, primary zoospores inactive, transforming *in situ* into group of cystospores soon after clevage and flagellation; sporangia 25—37 by 25—32 μm; discharge tube 7.5—20 μm long; antheridia diclinous

P. nigrescens sp. nov.

The author wishes to thank mgr Kazimierz Nowak for preparation of Latin diagnose.

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Nowy gatunek *Pythiogeton* odznaczający się nieaktywnymi pierwotnymi zoosporami

Streszczenie

Praca zawiera opis *Phythiogeton nigrescens* sp. nov., grzyba wyizolowanego na gałązki i obumarłe owoce *Sorbus intermedia* Pers. z próby wody i piasku pobranego z nadwiślańskiej plaży w Warszawie.