



HOKKAIDO UNIVERSITY

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A NEW SPECIES OF PYTHIUM PARASITIC ON *AEGAGROPILA SAUTERI* (NEES) KÜTZING

BY

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(with Two Text-figures)

A considerable number of fungi have been found to be parasitic on algae, but nothing has been reported of pythiaceous fungus parasitic on *Aegagropila* up to the present time. *Aegagropila Sauteri* (NEES) KÜTZING, a ball-shaped green alga, which attracts popular attention on account of its remarkable form and of the limited distribution, is often affected by some parasitic fungi. The writer found a species of *Pythium* parasitic in the filaments of this alga collected in Lake Akan, Hokkaido. The present paper is written to report the Aegagropila-disease together with the taxonomy of the fungus.

The writer wishes to express here his sincere thanks to Prof. Emer. K. MIYABE for his valuable suggestions and to Profs. S. ITO and Y. TOCHINAI for their kind directions. He is also indebted to Mr. Y. ABE, a forest ranger in Akan district, for his kindness in collecting the materials and giving them to the writer.

The affected plants become soft and hairy in appearance and their surface filaments etiolate to clayish yellow in color. The filaments are brittle and fragile, and fall off from the algal ball. The rotting progresses gradually into the centre, then the ball decreases in size, and breaks up into pieces finally. Under the microscope it is revealed that the affected filaments contain fine hyphae and yellowish oogonia. Sometimes it is observable that the vesicles develop at the tip of exit tubes which penetrate the cell wall of the host and that the zoospores differentiate in the vesicle. The zoospores swim actively in water surrounding the alga and settle on the wall of the filament. The cytoplasm and chloroplasts in the algal cells are killed. The hyphae penetrate the cross walls and spread from cell to cell towards the basal part of the filaments.

The fungus was purely isolated on artificial media for the sake of detailed observations. A small piece of the filament in which the fungus was growing actively was cut off with a scalpel and washed several times in sterile water.

Then it was placed on the surface of rice-grain decoction agar in a PETRI-dish. The dish was laid in a cool place and inclined to some degree to let water

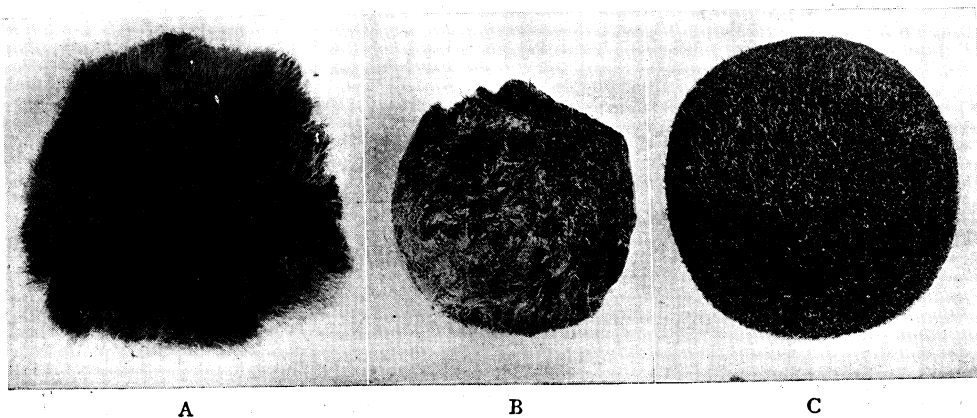


Fig. 1

Aegagropila Sauteri, infected (A & B) and healthy (C). ca. $\times \frac{2}{3}$

A. Photographed in water. B & C. Photographed in air.

flow down. When the fungus grew on the medium, a bit of hyphae was cut off from the margin of the mycelial mass and transferred to another dish.

The fungus grew well on the various media, plant-decoction agars and synthetic agars, except the strongly acidic media as apricot-juice agar. In the plate culture, the colonies were entire in the margin and sometimes formed concentric rings. The aerial hyphae developed vigorously in cottony appearance on several media and completely filled up the cavity of the test-tube in the case of the cultures on onion decoction agar slant. Although no reproductive organs were borne on the agar media, when a bit of hyphae was transferred into fresh water the sporangia and zoospores were formed abundantly.

The pathogenicity of the fungus to various algae was tested. The experiments were carried out in two series. In the first series a piece of the algal filament bearing actively living hyphae was used as the inoculum, while in the second series a bit of hyphal mass cultured on rice-grain decoction agar was used. In each series, two PETRI-dishes of four inches diameter containing about 20 c.c. of sterile water were prepared for each alga, and a small mass of algae was put in the water. One of two dishes was infested by the fungus and the other dish being non-infested was used as the control. Each dish was examined under microscope every day for a fortnight after the inoculation.

The present experiments were carried out from September to October in the year of 1931. The species of algae inoculated in these experiments and their habitat are as follows:—

1. *Oscillatoria* sp. (Maruyama, near Sapporo)
2. *Spirogyra* sp. A (Sapporo)
3. *Spirogyra* sp. B (Lake Shikotsu)
4. *Zygnema* sp. (Lake Shikotsu)
5. *Hydrodictyon reticulatum* (L.) LAGERH. (Lake Shikotsu)
6. *Draparnaldia* sp. (Maruyama, near Sapporo)
7. *Oedogonium* sp. A (Maruyama, near Sapporo)
8. *Oedogonium* sp. B (Lake Shikotsu)
9. *Cladophora* sp. (Lake Akan)
10. *Aegagropila Sauteri* (NEES) KÜTZING (Lake Akan)
11. *Sphaeroplea annulina* (ROTH.) AG. (Lake Shikotsu)
12. *Vaucheria sessilis* (VAUCH.) DC. (Maruyama, near Sapporo)

Among these algae, *Aegagropila Sauteri* was growing healthily in the laboratory for about two years. The other were collected in August to September of 1931 and were growing actively in the laboratory.

As the results of the experiments, no infection took place on the algae except two species, *Cladophora* sp. and *Aegagropila Sauteri*, in both series. *Cladophora* sp. was infected by the inoculation of affected algal filaments, while it was nonsusceptible to the isolated fungus. In the infected filaments, the hyphae grew in the cells and spread from cell to cell, but no oogonium formation was observed until the alga was completely destroyed. *Aegagropila Sauteri* was infected by the fungus in both series of the experiments. The infected alga showed apparently similar symptoms to those in nature. Though the alga had been destroyed before the oogonia matured, they were easily observed in the affected cells. In the present experiments, there was failure to infect the algae other than the two species mentioned above, but it is necessary to study further to determine the pathogenicity of the fungus to other algae. As an appendix to the present inoculation experiments, rice-seedlings were inoculated by the fungus, but the result was quite negative.

Finally, the fungus was examined from the taxonomic standpoint. There are many species of *Pythium* parasitic on green algae. *Pythium angustatum* SPARROW parasitic on *Vaucheria* sp. is most closely related to our fungus but it differs from the present species in the characters of oospore and in the number of antheridium. In our fungus the oospore is usually larger than that of *Pythium angustatum* and it is formed completely occupying the oogonium,

and only one antheridium attaches to one oogonium. Moreover, our fungus differs in the cultural characters from SPARROW'S fungus which forms oogonia abundantly on corn-meal agar. After a thorough literature study the writer came to the conclusion that the fungus under consideration has been hitherto undescribed and is a new species of *Pythium*. He proposes a new name *Pythium akanense* for the fungus. The technical description of the species is given here.

***Pythium akanense* TOKUNAGA, sp. nov.**

Mycelio entophyto; hyphis incoloratis, ramosissimis, plerumque ex cellula non exeuntibus, $0.8-2.4\ \mu$ crassis. Sporangii filiformibus, simulate hyphae similibus; tubulis exitus longis, membranam cellulae hospitis penetrantibus, infra membranam valde turgescitibus; vesiculis sphaeroideis vel ellipsoideis, 2-40 zoosporas gignentibus; zoosporis reniformibus, in parte concava binis ciliis praeditis; oogoniis semper in cellulis hospitis evolutis, $14.4-18.4\ \mu$ diam., membrana levi, tenuissima; antheridiis originis dubiae singulis, clavatis vel oblongatis, minutis; oosporis solitariis, sphaericis, $14-18\ \mu$ diam., in medio globulum grandem continentibus, perfecte oogonium implentibus, episporio levi, flavo, usque ad $0.8\ \mu$ crasso; germinatione nondum observata.

Hab. parasitice in cellulis *Aegagropilae Sauteri* (NEES) KÜTZING.

Hokkaido: Prov. Kushiro; Lake Akan (Y. ABE, Aug. 1931).

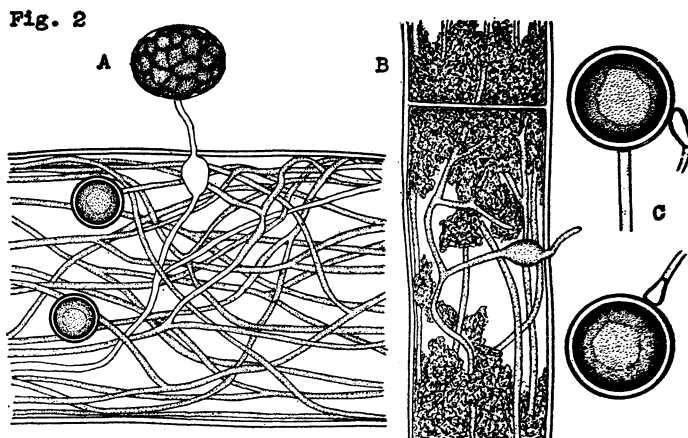


Fig. 2

Pythium akanense sp. nov.

A and B. Showing the fungus development
in host cells. $\times 430$

C. Oogonia with an antheridium. $\times 850$

Mycelium well developed in the filaments of the host alga passing from cell to cell through the cross walls; hyphae colorless, richly branched, $0.8-2.4\mu$ in breadth. Sporangia filamentous in shape, not differing in any way from the vegetative hyphae; exit tubes extending into the surrounding water penetrating the cell wall, but not going further from the substrata, similar to the hyphae but swollen very much just under the cell wall; vesicles spheroidal or ellipsoidal, irregular in size, producing 2-40 zoospores. Zoospores kidney-shaped, with two cilia near the hilum. Oogonia always formed in the host cells, usually terminal, spherical, $14.4-18.4\mu$ in diameter; their wall smooth, very thin. Antheridia coming from the doubtful origin, single to an oogonium, clavate or oblong, small in size. Oospores single, completely filling the oogonium, spherical, $14-18\mu$ in diameter, containing a central reserve globule which is irregular in shape; epispore smooth, yellowish in color, not very thick, up to 0.8μ in thickness; germination not yet observed.

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摘 要

毬藻に寄生せるピシウム属の一新種

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淡水産藻類に寄生せるピシウムは、従来屢々報告せられたるも毬藻に寄生せるものは未だ発見せられざるが如し。著者は北海道阿寒湖に於て採集せられたる毬藻に寄生せる一種を得たるを以て此處に報告せん。す。

菌に胃されたる毬藻は柔軟にして毬状を呈しその表面は黄白色に變ず。變色部は容易に脱落し腐蝕は漸次中心に進み、球は次第に小となる。被害部を檢鏡せば細き菌絲及び黃色なる藏卵器を細胞中に認め得べし。本菌を分離培養せしに酸度中性に近き寒天培養基上には極めて良好なる發育を成せり。寒天培養基上には如何なる繁殖器管をも生ぜざりしも、菌絲の一部を取りて水中に置くときは游走子を多數生ぜり。尙自然狀態に於ける菌及び分離せる菌絲を接種源とし、本菌の種々なる藻類及び稻に對する病原性を調査せしに *Cladophora* sp. 及び毬藻を除きては全く陰生に終れり。本菌を従來發表せられたる同属の各菌と比較するに、之と一致せる種類無きを以て著者は之を新種と認め *Pythium akanense* TOKUNAGA と命名し記載文を掲げたり。

終に臨み材料の蒐集に援助を與へられたる安部與市氏に感謝の意を表す。