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Sclerotinia pirolae: sclerotial ontogeny and occurrence in Finland

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Sclerotinia pirolae Grosse (Ascomycetes: Helotiales), an ovaricolous, sclerotia-forming fungus, is reported as new to Finland on *Pyrola chlorantha* Swartz, *P. media* Swartz, *P. minor* L., *P. minor* × *norvegica*, *P. minor* × *rotundifolia*, *P. norvegica* Knaben and *P. rotundifolia* L. It has also been found on *P. minor* × *norvegica* and *P. norvegica* from the adjacent Soviet Karelia. No ascocarps were discovered, but its sclerotial morphology suggests that the species probably belongs to the genus *Monilinia* Honey. The sclerotial stroma includes suscept tissues of the host and is of the hollowspheroid type characteristic of *Monilinia*. The sclerotial ontogeny of the species is described and illustrated.

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Sclerotinia pirolae

Sclerotinia pirolae Grosse, Ann. Mycol. 10: 388. 1912. — *Stromatinia pirolae* (Grosse) Naumov (in Kursanov, Opredel. Nizh. Rast. 3:379, 1954; comb. inval.) Fl. grib. Leningr. obl. 2:136, 1964.

Sclerotinia pirolae was described from Latvia, Estonia, Byelorussia and the Leningrad Region, where it was reported as a frequent parasite in the ovaries of *Pyrola rotundifolia* L., *P. minor* L., *P. chlorantha* Swartz, *P. media* Swartz and *Moneses uniflora* (L.) A.Gray (Grosse 1912, Bucholtz 1916, Bucholtz & Grosse 1917, Lippmaa 1928). This species has probably not been reported from outside the Soviet Union. In any case it has often been overlooked by mycologists, Kohn (1979a) listed it among imperfectly known taxa, for which she had not obtained any material.

Examination of the specimens of *Pyrola* and its allied genera *(Moneses, Orthilia, Chimaphila)* in the Finnish herbaria (H, JYV, KUO, OULU, TUR) has revealed that it occurs in Finland, too. Its presence was studied only under a dissecting microscope and most of the herbarium specimens had been collected when they were flowering, i.e. when the fungus is still so immature that it is difficult to recognize. It seems to be widespread in Finland, but it possibly avoids the southwestern part of the country. The fungus was not detected in any specimens collected outside Finland and the Soviet Union.

Specimens examined:

On *Pyrola rotundifolia:* **Finland.** *Uusimaa.* Helsinki, Mellunkylä, 1980 Hämet-Ahti 2858. (H). Espoo, Bredviken, 1964 Gyllenberg (H); Ollas, 1980 Ahti (37964) & Slack (H). Vantaa, Iso-Bastö, 1930 Paalanen (OULU). Mäntsälä, SW part, 1963 Suominen & Kytövuori (H); Mäntsälä village, 1954 Korhonen (H). — *Satakunta.* Eura, Vähäjärvi, 1973 Kause & Seikkula (OULU). — *Etelä-Häme.* Nokia, Pitkäniemi, 1932 Heikinheimo (H). Lammi, Palonen, 1980 Ahti 39201 (H). — *Etelä-Savo.* Joutseno, Vesikkola, 1944 Ikkala (H). Lappeenranta, Vihtola, 1980 Vuokko (H). — *Etelä-Pohjanmaa.* Alavus, Lahdenkylä, 1930 Railonsala (H). — *Kainuu.* Puolanka, Väyrylänkylä, 1966 Ulvinen (OULU). — *Koillismaa.* Kuusamo, Tyränkijärvi, 1967 Pyykkö (H).

On *Pyrola minor:* **Finland.** *Uusimaa.* Sipoo, Immersby, 1980 Ahti 39212 (H). — *Etelä-Häme.* Janakkala, Rehakka, 1956 Vilpa (OULU). Lammi, Palonen, 1980 Ahti 39201 (H). — *Pohjois-Häme.* Virrat, Toisvesi, 1964 Kytövuori (H). — *Kittilän Lappi.* Kittilä, Aakenus, 1963 Visa (H), Tepsa, 1966 Rintanen (OULU).

On *Pyrola minor* × *norvegica:* **U.S.S.R.** *Karelian A.S.S.R.* Louhi, Päänuorunen, 1942 Söyrinki (OULU).

On *Pyrola minor* × *rotundifolia:* **Finland.** *Satakunta.* Merikarvia, 1962 Suominen (H). — *Etelä-Häme.* Ruovesi, Mustajärvi, 1969 Söyrinki (OULU).

On *Pyrola norvegica:* **Finland.** *Koillismaa.* Kuusamo, Paljakka, 1980 Ahti 37962 (H). — *Kittilän Lappi.* Kittilä, Kuivasalmi, 1961 Rintanen (H). Muonio, Olostunturi, 1922 Elfving (KUO). — **U.S.S.R.** *Karelian A.S.S.R.* Louhi, Kutsajoki, 1936 Sonck (H).

On *Pyrola chlorantha:* **Finland.** *Satakunta.* Karkku,

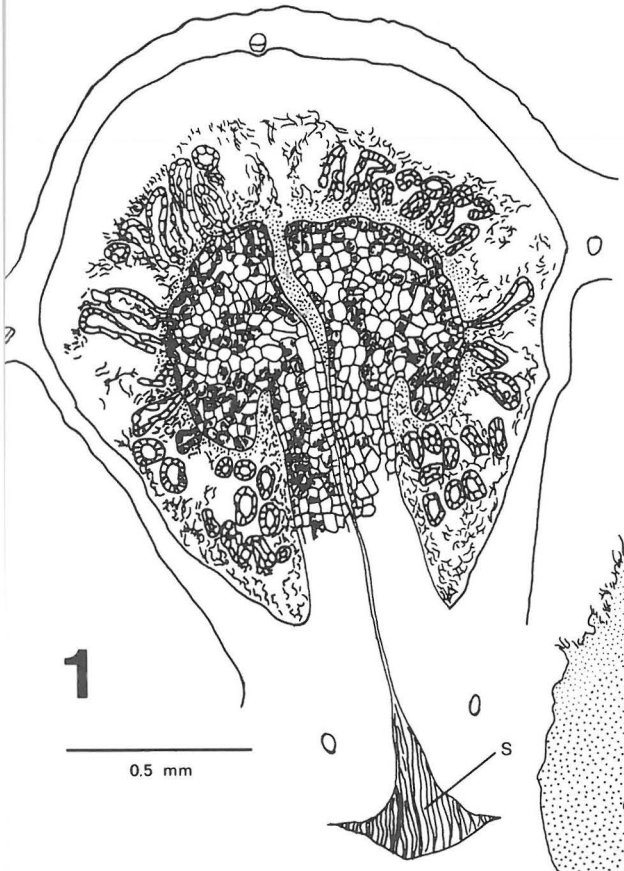
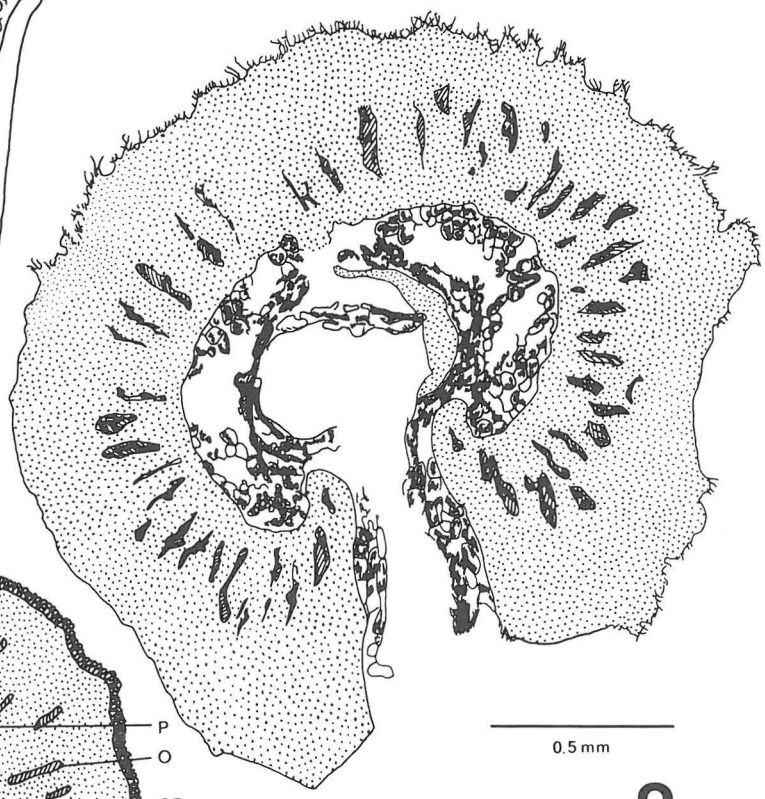
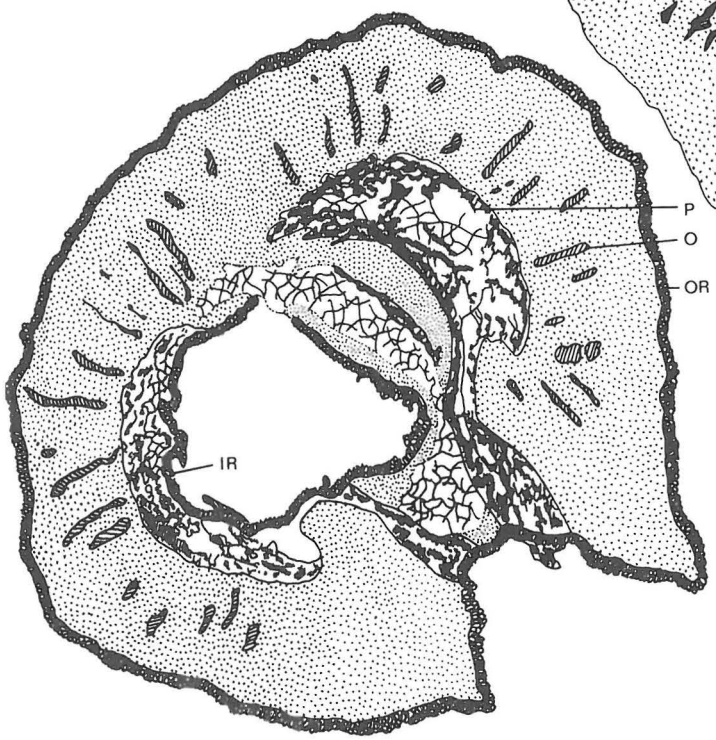


Fig. 1. *Pyrola rotundifolia*. Transverse section of an ovarial locule with invading hyphae. Compact inner part of the developing stroma stippled. — S: stylar canal with mucilaginous content.

Fig. 2. *Sclerotinia pirolae*. Transverse section of the developing stroma at the lilac stage. Ovules (hatched) embeded among the compact hyphae. Host: *Pyrola rotundifolia* × *minor*.

Fig. 3. *Sclerotinia pirolae*. Transverse section of an overwintered stroma from a capsule of *Pyrola rotundifolia*. — OR: outer sclerotial rind, IR: inner sclerotial rind, O: remnants of ovules, P: remnants of the placenta.





Pakkala, 1880 Wegelius & Hjelt (OULU). — *Etelä-Savo.* Sulkava, Partala, 1970 Miettinen (H).

On *Pyrola media:* **Finland:** *Uusimaa.* Sipoo, Hindsby, 1980 Ahti 39220 (H). — *Etelä-Häme.* Pirkkala, 1908 Sola (H).

The host species in this material are *Pyrola chlorantha, P. media, P. minor* and *P. rotundifolia,* which have been reported previously (e.g. Grosse 1912), and *P. minor* × *norvegica, P. minor* × *rotundifolia* and *P. norvegica* Knaben, which are new hosts. *Moneses uniflora* (*Pyrola uniflora* L.) was not confirmed to be a host in Finland, though the plant has been seen growing very close to an infected *P. rotundifolia* colony. *Orthilia secunda* (L.) House (*P. secunda* L.) occurs among infected colonies, too, but it remains uninfected.

S. pirolae forms sclerotia in the capsules of the host and prevents the development of the seeds. The size of the black, mature sclerotia in the present material is 1.9—3.5 × 1.0—2.6 mm. The dimensions reported by Grosse (1912, Bucholtz & Grosse 1917) and Naumov (1964) are 2.5—4 × 1.5—2.5 mm. The largest sclerotia were found on *Pyrola media* but otherwise there seems to be no correlation between the host species and the size of the sclerotia. The form of the sclerotia copies the form of the loculus: they are ovoid to trigonous with two straight to concave sides and one convex side.

The number of sclerotia per capsule in the herbarium specimens is 1 to 5; several specimens have only one sclerotium per capsule. The number of infected flowers per inflorescence is often 1 to 3, rarely 5 or more. The field observations in 1980 revealed that in two fairly large infected colonies of *Pyrola rotundifolia* in Helsinki (specimen Hämet-Ahti 2858) there were 4—8 infected flowers in an inflorescence and usually 4—5 sclerotia per capsule. In this material the infected flowers are usually in the middle part of the inflorescence. The preference of the lowest part of the inflorescence reported by Grosse (1912) was not observed.

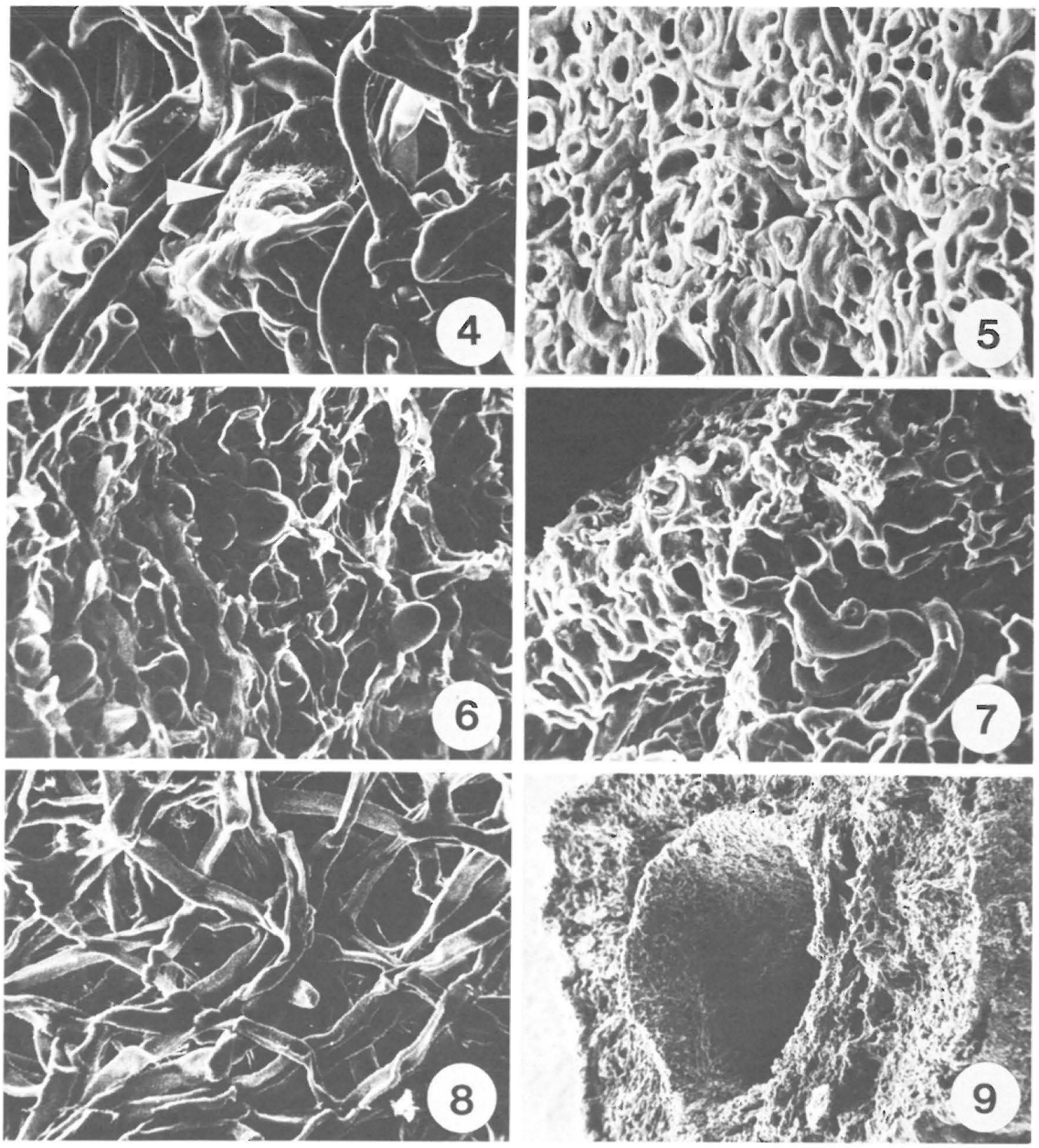
According to Grosse (1912, Bucholtz & Grosse 1917), the sclerotium of *S. pirolae* is biennal: it is full-grown after the first summer, overwintering in the capsule, is shed during the second summer and germinates only in the third summer. In southern Finland the sclerotia seem to remain in the capsule up to the end of July. The one-year-old sclerotia did not germinate in moist chamber cultures even when they were kept in —18°C for one week before the experiment. No ascocarps were found by the infected colonies, though searched for carefully through spring and summer.

This fungus has hardly any visible effect on its host other than that it destroys its seeds. It is not systemic (confirmed by sectioning pedicels and stems below infected capsules). This agrees with the observations of Grosse (1912, Bucholtz & Grosse 1917). The locule containing a sclerotium may look fuller than the others but this is not always the case. The rose-red colour of infected ovaries reported by Bucholtz and Grosse (1917) and by Lippmaa (1928) does not seem to be very significant. It is easy to recognize the presence of the sclerotia because infected capsules feel harder when pressed between the fingers than the healthy ones. The presence of black sclerotia is also easily seen in open mature capsules, both in autumn and in spring (then on dead stems).

Before Grosse (1912) described this fungus, it was reported without a name by Rothert (1900) from Latvia and by Bucholtz (1900:151) from Latvia, too. Whetzel (1945), who erected the family Sclerotiniaceae, used the stroma type as a major character in delimiting new and revised genera. According to him and subsequent authors, the tuberoid sclerotia of *Sclerotinia* Fuck.s.str. are formed free on mycelium, and are devoid of remnants of suscept tissue, while the discoid sclerotium of *Ciborinia* Whetzel is characterized by the inclusion of undigested vascular elements and other host debris within the stroma. The sclerotium of *S. pirolae* includes suscept tissues and therefore resembles that of *Ciborinia* rather than *Sclerotinia,* but, as also mentioned by Bucholtz & Grosse (1917), the sclerotium of *S. pirolae* has a clear central cavity covered by a thin black rind. This means that the stroma is of the hollow-spheroid type, characteristic of species of *Monilinia* (Whetzel 1945:668). In fact, *S. pirolae* may actually belong to *Monilinia,* which causes mummified fruits in many species of Ericaceae, a family close to Pyrolaceae. As suspected by Kohn (1979a), it can hardly belong to *Stromatinia* (Boud.) Boud., although it was placed in that genus by Naumov (e.g. 1964). However, as we have not seen the ascocarps, we will refrain from making any new combination.

Sclerotial ontogeny

Three stages have been distinguished in studying the formation and anatomy of the sclerotia: initiation, development, and maturation. Formation of the sclerotial initial begins in the ovary at full anthesis. Ascospores are probably disseminated by air currents to the stigma because infective hyphae were found in the stylar and placental canals as well as in the locules of the ovary (Fig. 1, see also Bucholtz & Grosse 1917). Successful invasion of the germ tube



Figs. 4—9. *Sclerotinia pirolae*. SE micrographs of the developing (4—6) and mature (7—9) sclerotium. — 4: Loosely arranged mycelial strands in outer parts of the medulla. Note the remnants of an ovule (arrow). — 5: Compact inner parts of the medulla. — 6: Developing inner rind with bulbous cells. — 7: A part of the outer rind (left) and the medulla of the overwintered sclerotium. — 8: Interwoven mycelium in the placenta. — 9: Transverse section of the mature sclerotium of the hollow-spheroid type. Host: *Pyrola rotundifolia*. — *Magnifications:* 4 — 8: × 1230. — 9: × 120.

of the ascospores evidently depends on the presence of glandular secretions produced by the stigmatic cells. After the spores have germinated, the mycelium grows rapidly along the surface of the stylar and placental canals to the epidermis of the placenta. The canals and the epidermis are covered with a glandular substance secreted by the lining cells shortly before fertilization, when the embryo sacs are already

mature (Pyykkö 1968: 158). Secretions may satisfy the food requirements of the invading hyphae.

The mycelial mat covering the surface of the placenta is compact and may contain interhyphal liquid, which Colotelo (1974: 1129) reported in the developing sclerotia of *Sclerotinia sclerotiorum* (Lib.) de Bary in culture. Between the ovules the mycelium is loosely arranged and with prosenchymatous, ramified, thin walled hyphae. The ovules are also frequently infected by hyphae and the mycelial strands appear to enter the embryo sacs through the micropyle.

It seems that after invasion the mycelium secretes a mucilaginous substance, which plugs the stylar canal (Fig. 1). No substance of this kind was seen in sections of uninfected ovaries.

After an ovarian locule has been completely invaded by the mycelium, a sclerotium is formed. It evidently grows by taking its nutrients from the living cells of the placenta and ovules. However, both the integuments and the placenta contain abundant cells with tannin (Pyykkö 1968), which the mycelium cannot utilize as food, and remnants of these tissues are seen even in the mature sclerotia (Figs. 2, 3). The integuments are easily recognized in the compact mass of fungal tissue by their location, form and characteristic colour.

At the early stage of development the sclerotia are light lilac; later they turn white. In these stages the exterior parts of the sclerotia are seen as a mass of loosely interwoven mycelial strands (Fig. 4) and the interior parts near the placenta are compact with rather thick cell walls (Fig. 5). No rind cells have formed yet on the outer surface of the sclerotia, but on the inner one, which surrounds the central cavity, bulbous hyphal tips occur between thin mycelial strands (Fig. 6). These are the future rind cells, though not yet pigmented. Many undigested placenta cells remain, but the integuments are all that is left of the ovules (Figs. 2, 4).

The structure of the mature overwintered sclerotium (Fig. 3) resembles that of *S. sclerotiorum* in many features (Kohn 1979a, 1979b). The medulla is composed of compact hyaline tissue with heavily gelatinized hyphal walls (2 to 4 μm thick). But, unlike the case in *S. sclerotiorum* (Colotelo 1974), the hyphae are not embedded in an amorphous matrix (Fig. 7). Resistant tissues of the integuments and placenta occur among the hyphae of the medulla. Remnants of the placenta are traversed by loosely arranged thinwalled hyphae (Fig. 8). The sclerotial rind layer consists of bulbous, dark-coloured, thin-walled cells (1 to 2 μm thick), which are the apices of the medullary hyphae. Pigmentation may occur in the walls of the two to three outermost cell layers. Rind cells are also evident on the inner surface of the sclerotia, i.e. adjacent to the longitudinal central cavity, which usually forms in only one of the apical lobes and in the basal lobe of the placenta (Figs. 3, 9).

The form of the sclerotium of *S. pirolae* is determined by the locule in which the sclerotium develops. In each locule of the ovary the *Pyrola* species have a characteristic massive, axile, U-shaped placenta (Pyykkö 1969), which together with numerous ovules fills the locule (Fig. 1). When the mycelial strands of the developing sclerotium finally surround the placenta and ovules completely, the sclerotium takes the shape of a large placenta without the stalk part.

The senior author is responsible for the anatomical studies and for the discussion on the development of the sclerotia, the junior author for the determination of the species and the ecological discussion.

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