Ascocoryne sarcoides and Ascocoryne cylichnium. Descriptions and comparison

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Descriptions are given and characters evaluated of apothecia and cultures of *Ascocoryne sarcoides* (Jacq. ex S. F. Gray) Groves & Wilson and *A. cylichnium* (L. R. Tul.) Korf. The separation into the two species may be justified by the occurrence of typical ascoconidia in *A. cylichnium*, by a relatively sharp boundary between ectal and medullary excipulum in *A. sarcoides*, and to some extent by other characters.

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Ascocoryne species have been isolated by many authors from wood in living spruce trees both in North America and in Europe. In Norway the *Ascocoryne* species seem to be more common than any other group of fungi in unwounded stems of *Picea abies*. They do not seem to cause any rot or discoloration of importance, but they may influence the development of other fungi in the stems.

Great confusion exists regarding identification of isolates of the *Ascocoryne* species. In a preliminary paper (Roll-Hansen & Roll-Hansen 1976) the authors reported isolation of *Ascocoryne sarcoides* (Jacq. ex S. F. Gray) Groves & Wilson and *A. cylichnium* (L. R. Tul.) Korf from stems of *Picea abies*. Having found allantoid conidia in *A. sarcoides* ascospore-cultures and ovoid conidia in *A. cylichnium* ascospore-cultures the authors based the identification of the cultures from the spruce stems on these criteria. But later we found both ovoid and allantoid conidia in varying proportions in some of the cultures. We therefore realized that a closer comparison between apothecia of *A. sarcoides* and *A. cylichnium*, and of ascospore-cultures from the same ascomata was necessary.

In the present paper we first present a short review of some literature data relevant to distinguishing between the two species. Afterwards our own material is treated, and a comparison based on literature and our own data is given.

Former descriptions

Ascocoryne sarcoides (Jacq. ex S. F. Gray) Groves & Wilson

Groves & Wilson (1967) gave the diagnosis of the genus *Ascocoryne* and transferred *Octospora sarcoides* Jacq. ex S. F. Gray (*Coryne sarcoides* [Jacq. ex S. F. Gray] Tul.) to that genus. The conidial state is *Coryne dubia* Pers. ex S. F. Gray (*Pirobasidium sarcoides* Höhn.). Descriptions of the apothecia with the asci and the ascospores have been given by, for example, Knapp (1924), Dennis (1956), Gremmen (1960), Christiansen (1962), and Jahn (1967). The apothecia are very similar to those of *A. cylichnium*. What Thind & Singh (1969) described as *Coryne cylichnium* (Tul.) Boud. is apparently *A. sarcoides*.

Apothecia and conidia-producing stromata in nature. – Knapp (1924) reported that the apothecia are 8–13 mm wide. Dennis (1956) stated that the apothecia are 2–10 mm across, the paraphyses unbranched, about 1 μm thick, either enlarged to 2 μm or abruptly swollen to 4 μm at the tip, and that the size of the asci is 90–120 × 8–10 μm.

The ascospores have been described as ellipsoid, 1–4-celled, and considerably smaller than in *A. cylichnium*. According to Knapp (1924) they are 12–18 × 4–5 μm; besides the normal ascospores Knapp also found egg-shaped conidia in the asci. Dennis (1956) gave the fol-

lowing description: 'Ascospores uniseriate or biseriate, elliptical or inequilateral, at first non-septate with 2 oil drops, ultimately 1-septate, occasionally with 3 septa, 10–15(–19) × 3–5 μ, sometimes germinating in situ by a terminal germ-tube'. Excipulum of '*C. cylichnium*' in his Fig. 151 fits *A. sarcoides* better than it fits *A. cylichnium*.

In the conidial state, *Coryne dubia (Pirobasidium sarcoides)*, some millimetres high, club-like, or often rather irregular, conidial stromata are formed. They have the same colour and consistence as the ascomata. The conidia are usually ca. 3.5–6 × 1 μm (Höhnel 1902; Knapp 1924; Dennis 1956; Christiansen 1962). Tulasne & Tulasne (1865) stated that ovate (3.5–6.5 × 3 μm) or sometimes rather globose conidia were also formed on the conidial stromata. Höhnel (1902) found, however, that these 'conidia' were partly pear-shaped 'basidia' producing the true conidia (4 × 1 μm), partly roundish cellulae from which the 'basidia' had grown out.

Cultures on malt agar. – Gremmen (1960) reported globular 'microconidia' in cultures from ascospores of *A. sarcoides*. He thus apparently obtained a type of conidia which is otherwise supposed to be characteristic of *A. cylichnium*.

Berthet (1964) found rod-shaped, slightly bent conidia, ca. 5 × 1.5 μm, on the surface in an agar culture, but ovoid or subspherical conidia, 2–4 × 2 μm in the agar. Delatour (1976) reported a similar dimorphism, but he did not mention whether the roundish conidia were formed in the agar substrate.

Etheridge (1957) found no reaction zone on gallic acid, except a very intense dark brown zone in one case. On tannic acid he found no reaction zone, or in some isolates light to dark brown zones.

Ascocoryne cylichnium (L. R. Tul.) Korf

Peziza cylichnium was described by Tulasne (1853) and transferred to *Coryne* by Boudier in 1907. The combination *Ascocoryne cylichnium* was validated by Korf (1971). It is interesting that Karsten (1885) did not consider it a separate species but named it *Coryne sarcoides *urnalis* (Nyl.) Karst. (*Sarcodea sarcoides *urnalis* (Nyl.) Karst. in Not. Sällsk. Faun. Flor. fenn. 11: 232, 1870; *Ombrophila sarcoides *urnalis* (Nyl.) Karst. in Myc. Fenn. 1: 87, 1871). Descriptions of the apothecia were given, for example, by Knapp (1924), Dennis (1956), Gremmen (1960), Christiansen (1962), and Jahn (1967).

Apothecia and indication of conidia-producing stromata in nature. – Knapp (1924) reported that the apothecia are 4–25 mm wide and that the ascospores are mostly 25–30 × 6–7 μm, spore minimum being 20 × 5 μm.

Dennis (1956) gave the following description: '*Apothecia* like those of *C. sarcoides* but often larger, up to 15 mm. across. *Flesh* of slender hyaline hyphae, about 1μ thick, loosely interwoven in a colourless gelatinous matrix. *Excipulum* 40–50 μ thick, of more or less isodiametric, thin-walled, angular cells, about 8–10 μ diameter, forming a compact parenchymatous tissue with an irregular outer surface. *Asci* cylindric-clavate, long-stalked, conical-truncate at the apex, the pore blue in Melzer's reagent, 8-spored, about 140–200 × 10–12 μ. *Ascospores* biseriate fusiform or inequilateral, equally pointed at each end, ultimately multiseptate and often abstricting spherical secondary spores, about 2 μ across, while still in the ascus; (14)–18–30 × 4–6 μ. *Paraphyses* slender, filiform, slightly enlarged towards the apex, embedded in colourless gelatine. On fallen logs and stumps (Fig. 151).' In the figure he drew ectal excipulum and part of medullary excipulum, paraphyses, ascus, ascospores, and spherical secondary spores; the border between ectal and medullary excipulum ('excipulum' and 'flesh') is very sharp, and the ascospores are acute at both ends.

According to the diagnosis given by Tulasne the ascospores are 22–26 × 5–6 μm and rounded at both ends.

In most descriptions no conidial state (except ascoconidia) has been mentioned. But Christiansen (1962) reported finds of apothecia of both *Coryne cylichnium (Ascocoryne cylichnium)* and *Coryne sarcoides (Ascocoryne sarcoides)* accompanied by *Pirobasidium sarcoides* Höhnel (*Coryne dubia* Pers. ex S. F. Gray). Usually *C. dubia* is accepted to be the conidial state of *A. sarcoides* only.

Cultures on malt agar. – Gremmen (1960) shortly described cultures of *A. cylichnium* on malt agar: 'Culture-work (No. 273): Cultures obtained from ascospores develop alike as *Coryne sarcoides*. In older cultures characteristic club-shaped excrescences up to 2.5 mm, producing 4 × 1.5 μ colourless 1-celled, more or less curved conidia, and reddish coloured microconidial pustules, were observed.' Gremmen ap-

parently happened to get a conidial state corresponding to *C. dubia* in his cultures from ascospores of *A. cylichnium*.

Some Norwegian collections. Descriptions of apothecia and cultures

Methods

Fresh apothecia from collections of *A. sarcoides* and *A. cylichnium* were placed into agar slant tubes or petri dishes to throw their ascospores upwards on sterile agar surfaces, and into moist chambers to throw them on clean, flamed slides. The ascospores on the agar surfaces provided the pure cultures. The ascospores on the slides were mounted in lactophenol-cottonblue for microscoping. In most cases 50 or 20 ascospores were measured. Some of the apothecia were fixed in 70% FAA, embedded in paraffin wax, microtomed 6 to 12 μm thick in ribbons, stained in safranin-fastgreen and mounted in canada-balsam (Johansen 1940). In addition fruitbodies from all collections were dried. Hand sections were made from the dry apothecia after moistening by wrapping them into wet filter paper. Sections from all collections were mounted in Melzer's reagent. If by this method the reaction to iodine was negative, then reaction after rehydration in 5% aqueous KOH solution was examined (Kohn & Korf 1975; Nannfeldt 1976). Some sections were mounted in lactophenol-cottonblue.

Also herbarium material was examined from which no cultures had been taken. The ascospores from these specimens were measured in hand sections of apothecia mounted in lactophenol-cottonblue.

The cultures described were polyascospore-isolates or sometimes (specially indicated) monoascospore-isolates. Culture medium was malt agar (1.25% Difco Bacto-Malt Extract + 2% Difco Bacto-Agar). The cultures were examined in different light and temperature conditions:

1. At 15 C and a 12 hour per day photoperiod (cool-white fluorescent light + near-UV light).
2. Constant temperatures in darkness.
3. Varying room temperatures at different daylight in the laboratory.

Phenoloxidase production was tested on gallic and tannic acid malt agar (Nobles 1948). Growth rate of the isolates was measured on malt agar in petri dishes in darkness at the respective temperatures. Margin of the mycelium (hypha ends) was marked on four diameters after a few days and again 8 to 10 days later. Conidia from cultures were measured in lactophenol-cottonblue under oil immersion (Objective Leitz 90 ×, n.a. 1.4).

Ascocoryne sarcoides

Material examined. – Four collections of apothecia, thrown-out ascospores, and ascospore-cultures from them were investigated thoroughly: one collection from *Malus* sp., two from *Picea abies*, and one from *Quercus* sp. Microtome sections were made from one of the collections from *P. abies*. In addition one ascospore-culture from apothecia on *Alnus* sp. and one from apothecia on *Betula* sp. were investigated thoroughly, but in these two cases material was lacking for further descriptions of the apothecia.

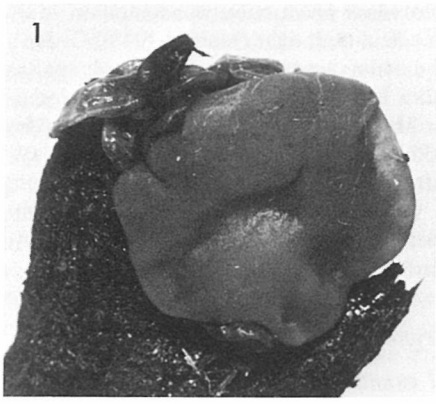
Apothecia from one collection on *Alnus*, three on *Betula*, and one on *Prunus* were also thoroughly studied, but no cultures had been taken. Apothecia from 15 other Norwegian collections from nine different hosts were sectioned and investigated more quickly.

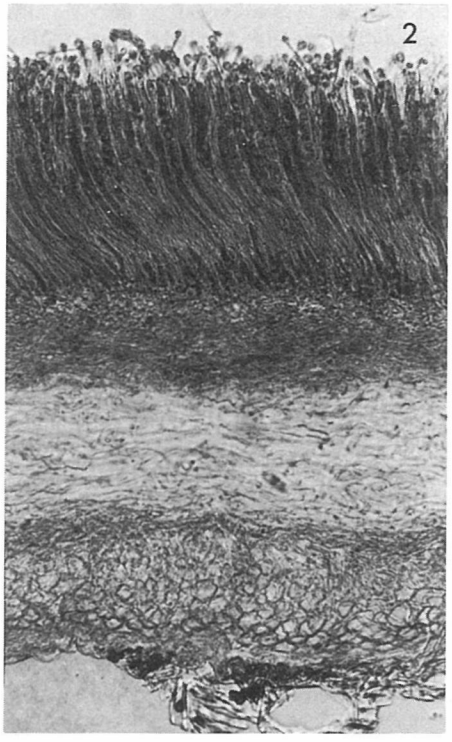
Apothecia. – In fresh material the diameter of the apothecia varied from 2 to 23 mm, the consistency was gelatinous, and the colour reddish purple (Fig. 1).

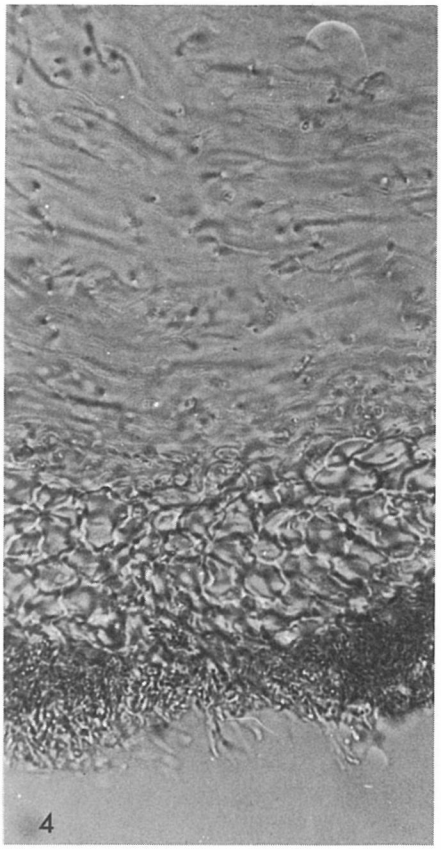
Ectal excipulum often consisted of two distinct sharply separated layers, but the outer layer was sometimes poorly developed or lacking.

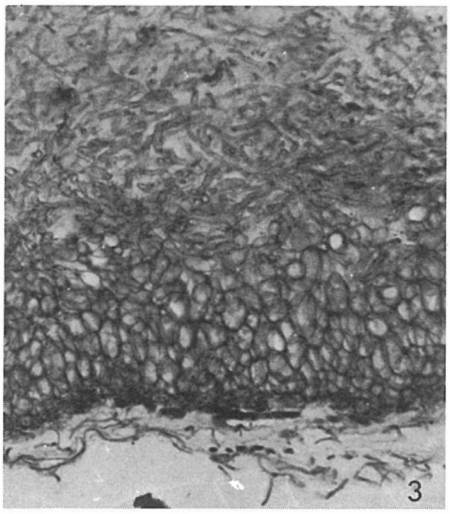
Outer layer of ectal excipulum was usually of textura intricata, mostly 0–30 μm thick, consisting of 1–4 μm thick hyphae in a gel (Figs. 2, 3); it easily weared off. Instead of the gelatinous layer there was sometimes found a loose layer of short hyphae ending in thin conidiophores forming conidia typical of *Coryne dubia*, ca. 4–5 × 1 μm (Fig. 4).

Inner layer of ectal excipulum was a 30–100 μm thick pseudoparenchyma, composed of swollen, roundish, or somewhat angular, isodiametric or nearly isodiametric cells, ca. 5–40 (50) × 4–20 μm (Figs. 2–7). The cells were small near the edge of the apothecium, larger and more specific farther from the edge (Figs. 6 and 7). It

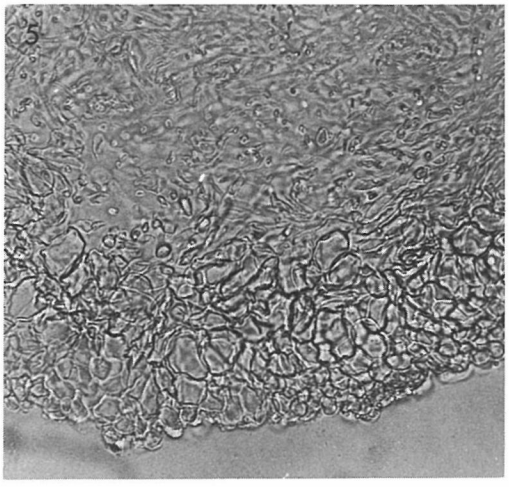


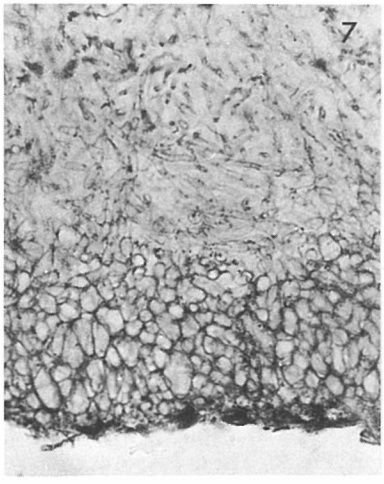


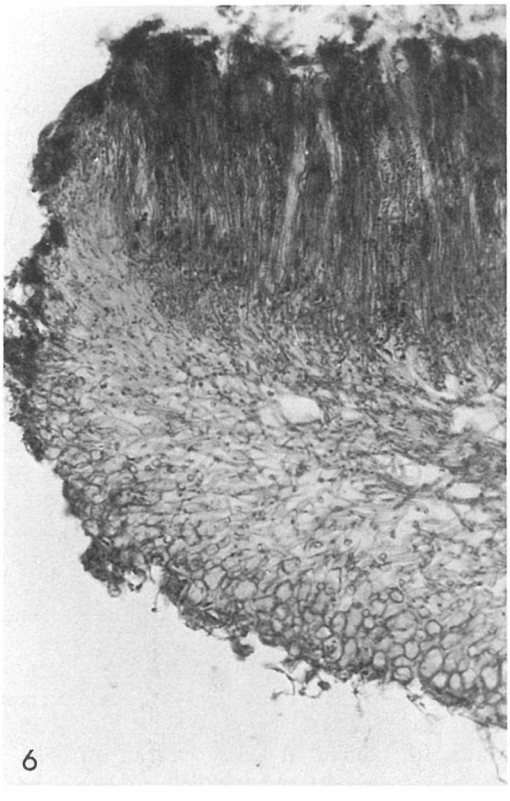


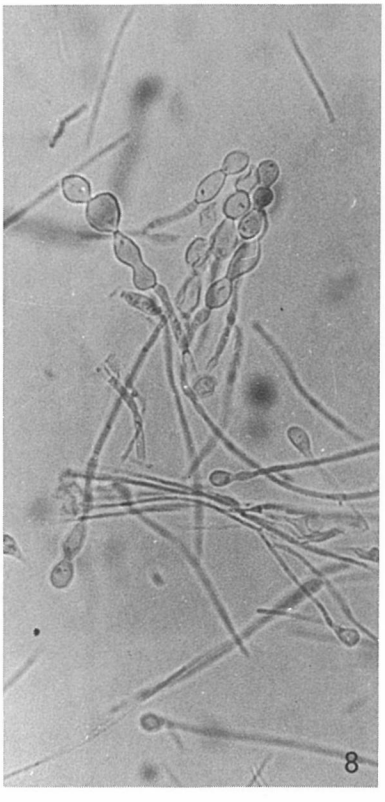


Figs. 1–4. *Ascocoryne sarcoides,* apothecia. Fig. 1. On *Malus* sp. ×3. Fig. 2. Median section, hymenium with asci and capitate paraphyses, hypothecium, medullary excipulum, ectal excipulum. Hand section in lactophenol-cottonblue ×250. Fig. 3. Median section, medullary excipulum, inner and outer layer of ectal excipulum. Microtomed 6 μm ×250. Fig. 4. Median section, outer layer of ectal excipulum with conidiophores. Hand section in Melzer's reagent ×500.









Figs. 5–8. *Ascocoryne sarcoides*. Fig. 5. Apothecium, median section of medullary and ectal excipulum. Hand section in Melzer's reagent × 250. Fig. 6. Apothecium, median section. Microtomed 12 μm × 250. Fig. 7. The same apothecium some distance from the edge, medullary excipulum, ectal excipulum's inner layer, and some rests of the outer layer. Microtomed 12 μm × 250. Fig. 8. Capitate and monilioid paraphyses. Squash slide in Melzer's reagent × 500.

was characteristic that the inner layer of ectal excipulum was rather sharply delimitated against the medullary excipulum.

Medullary excipulum of textura intricata. Hyphae 1.5–5(8) μm thick in a gel (Figs. 2–7).

The paraphyses were unbranched or branched, 1.5(2) μm thick. The tip was slightly to strongly swollen, in some collections only 2.0–2.5 μm, in others capitate and to 6.7 μm in diameter (Figs. 2, 8). Paraphyses with strongly swollen tips were sometimes monilioid (Fig. 8).

The asci were 85–130 × 6.5–10 μm (Fig. 9), in Melzer's reagent with blueing tips after rehydration in 5% aqueous KOH solution, but not always after rehydration in pure water.

Normal ascospores were 10.1–23.9 × 3.4–6.1 μm (Fig. 9). The ascospores from seven separate collections varied as shown in Table II as to mean of length and width, and as to minimum and maximum of length and width. The ascospores were 0–3-septate, sometimes 0–1-septate. The ends were mostly rounded, but in some collections more acute (Fig. 10). Abnormal, poorly developed ascospores were also found (Fig. 11). In one collection from *Quercus* sp. there were in addition to normal ascospores also shorter, elliptic ones with all transitions to spherical spores, 5–7 μm in diameter. Normal and abnormal spores occurred together in one ascus. Ascospore-culture was obtained also from this specimen.

Chains of small, roundish or elongated cells have been found in some asci (Figs. 11, 12). The chains may have been formed by germination of ascospores, but the appearance often indicated that they were primary – a kind of abnormal, degenerated ascospores, kept together by remains of cytoplasma. The small, roundish cells did not separate easily.

Cultures. – Cultures were compared on malt agar. The colour was first white, later usually lighter or darker violet or greyish-violet.

The hyphae were septate, 1–6 μm in diameter. Coils of hyphae were sometimes formed. Incrusted hyphae were seen in some cultures. In all cultures conidiophores occurred on free hyphae in the mycelial mat. Sometimes also conidial stromata were formed (Fig. 13).

In the mycelial mat the conidiophores were usually branched, often *Penicillium*-like and sometimes very complex (Fig. 14). The conidia were hyaline and one-celled of varying shape, usually allantoid or rod-shaped, 3.0–6.0(7.5) × 0.8–1.4(1.9) μm; but shorter, more roundish conidia, 2.0–3.0 × (0.8)1.2–1.6 μm, and all intermediates were often found. We did not find any difference of importance between the conidia formed by air mycelium and those formed submersed in the agar medium. The conidia swelled before they germinated, becoming broader, ellipsoid, usually measuring 4–7 × 2.5–5.0 μm.

The growth rate of ascospore-isolates from three collections was 0.7–1.2 mm per 24 hours on malt agar at 15 C.

On gallic acid malt agar reaction was lacking and the radial growth was 1–3 mm in seven days. On tannic acid malt agar the reaction was weak to moderately strong (++ to +++) and the radial growth was 1–5 mm in seven days.

Ascocoryne cylichnium

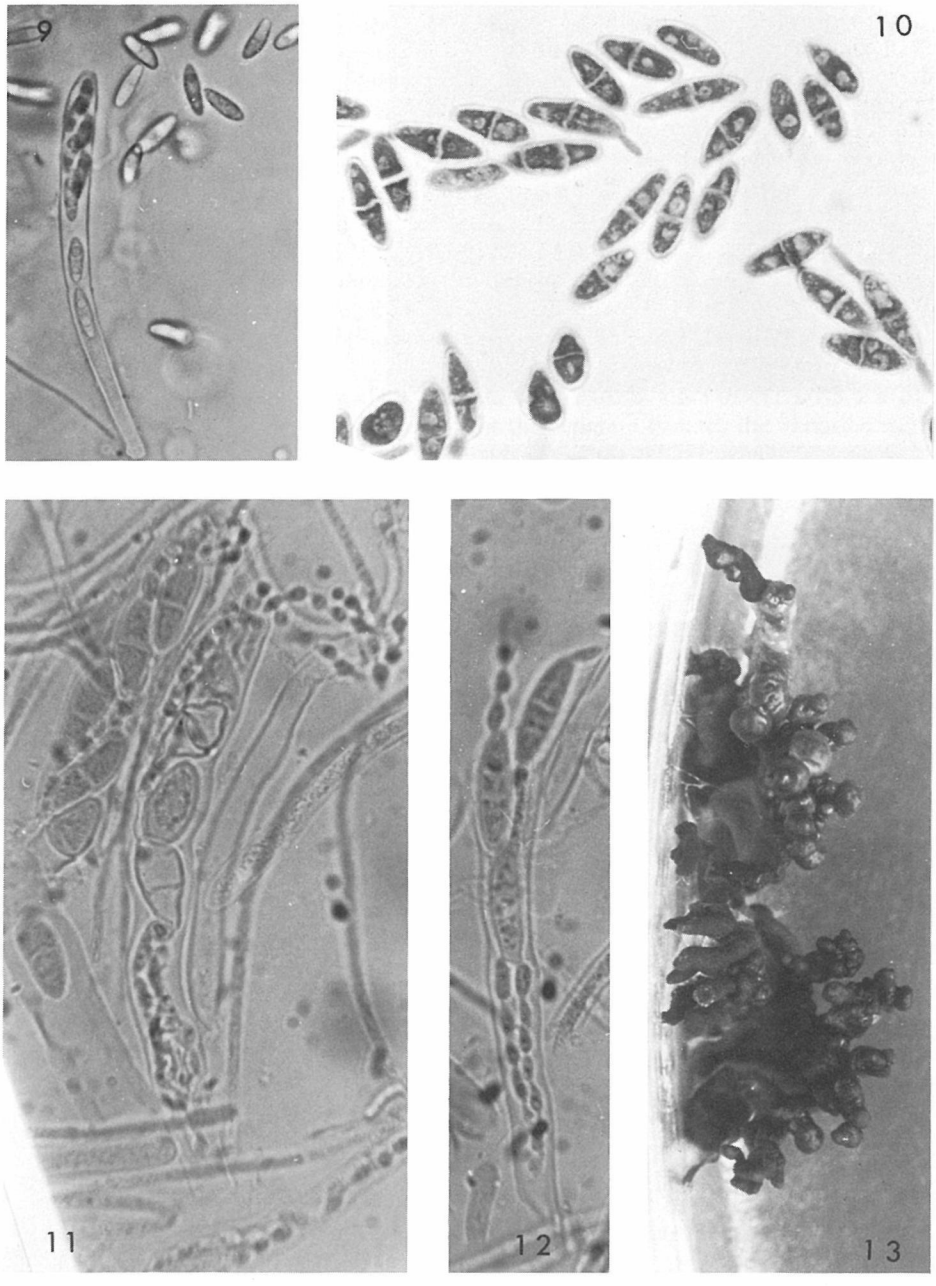
Material examined. – Sixteen collections of apothecia (three from *Betula* sp. and thirteen from *Picea abies*), thrown-out ascospores, and ascospore-cultures from them were investigated thoroughly. Microtome sections were made from seven of the collections from *P. abies*. Apothecia from 39 other Norwegian collections from nine different hosts were hand-sectioned and investigated more quickly.

Apothecia. – In fresh material the diameter of the apothecia varied from 1.3 to 12 mm; they were gelatinous and reddish-purple (Fig. 15), like those of *A. sarcoides*. As indicated by the epithets '*cylichnium*' and '*urnalis*' there might be a tendency that young apothecia were more cup-shaped in *A. cylichnium* (Figs. 16, 17) than in *A. sarcoides*, where the disc was usually rather flat (Fig. 1).

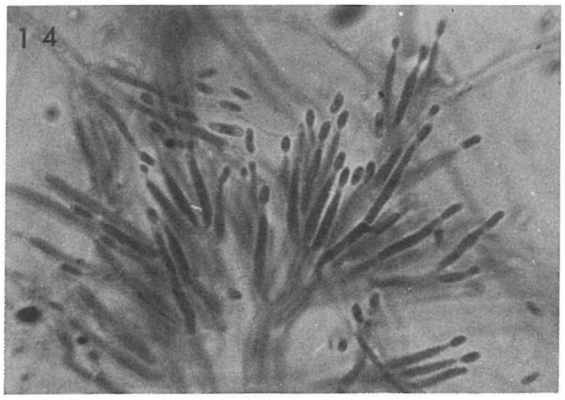
Ectal excipulum consisted in typical cases of an outer and an inner layer sharply separated from each other.

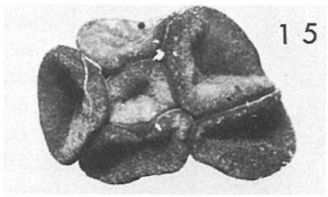
Outer layer of ectal excipulum was of textura intricata, mostly 0–30 μm thick, consisting of 1–4 μm thick hyphae in a gel. It was not always found. It easily weared off (Fig. 18). Conidiophores and conidia were produced from the outer layer of an apothecium in a polyascospore-culture on malt agar; in this case the conidia were both short, roundish, 2–3 × 1.3–1.8 μm, and allantoid, 3.5–4.5 × 1.0–1.5 μm, with transitions between the two types.

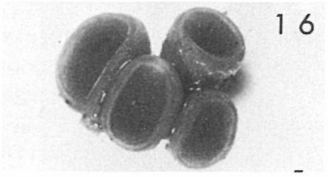
Inner layer of ectal excipulum (Figs. 18, 19) was usually 30–100 μm thick, composed of more or less swollen, isodiametric or more elongated

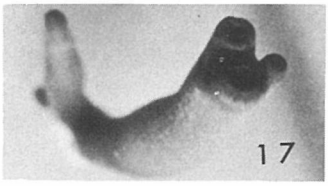


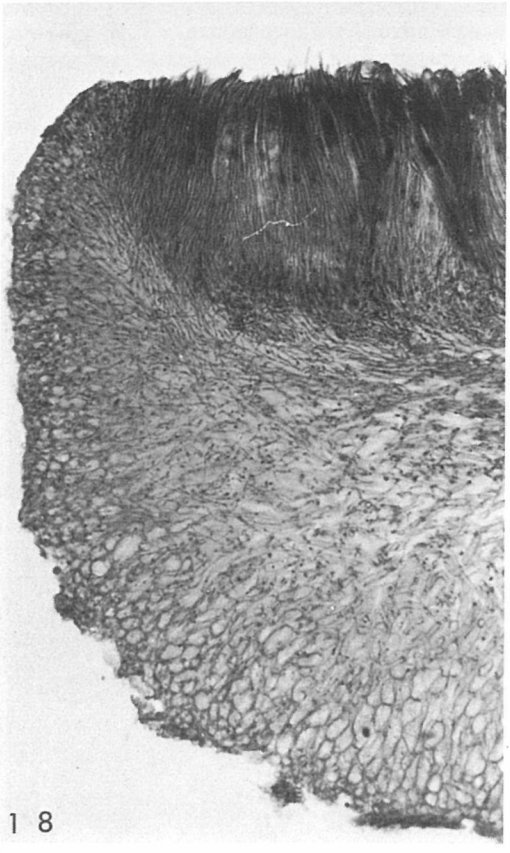
Figs. 9–13. *Ascocoryne sarcoides*. Fig. 9. Ascus and ascospores. Hand section in 50% lactic acid ×500. Fig. 10. Ascospores thrown out from an apothecium × 1,000. Fig. 11. Asci with normal and abnormal ascospores and chains of small, roundish cells. Squash slide in Melzer's reagent × 1,000. Fig. 12. Ascus with normal ascospores and chains of smaller cells. Squash slide in Melzer's reagent ×1,000. Fig. 13. Conidial stromata in culture on malt agar ×6.

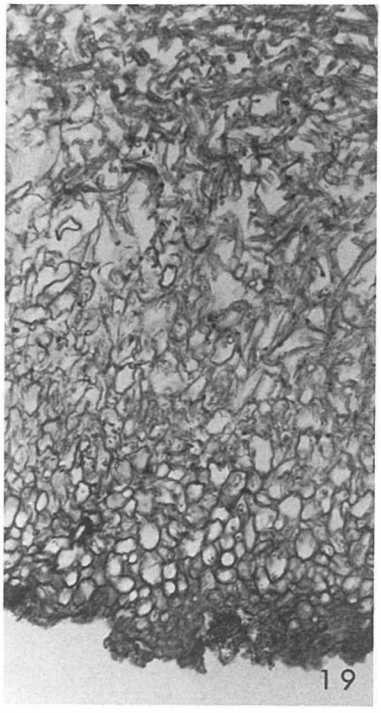












Figs. 14–19. Fig. 14. *Ascocoryne sarcoides,* conidiophores and conidia from culture on malt agar. Mounted in lactophenol-cottonblue ×1,000. Figs. 15–19. *Ascocoryne cylichnium.* Figs. 15–16. Two different collections of very small apothecia on *Picea abies.* Both gave ovoid to allantoid conidia in culture ×6. Fig. 17. Apothecium formed in culture on malt agar. Ascospore-isolate from the same collection as in Fig. 16 ×6. Fig. 18. Median section, rather swollen cells in the inner layer of ectal excipulum. Transition zone to medullary excipulum relatively narrow. The ascospores gave cultures with both ovoid and allantoid or rod-shaped conidia (cf. Fig. 28). Microtomed 12 μm×250. Fig. 19. Median section 2.8 mm from the edge. Transition zone to medullary excipulum broad and indistinct. The ascospores gave cultures with only ovoid conidia (cf. Fig. 27). Microtomed 6 μm ×250.

cells; there was a fairly even transition to the medullary excipulum. The cells near the edge of the apothecium were smaller than those farther from the edge (Figs. 18, 19). They were all transitions from apothecia with rather swollen cells in the inner layer of ectal excipulum and with a rather narrow transition zone (Fig. 18) to apothecia where swelling was weak and the transition zone broad and indistinct (Fig. 19). The last-mentioned extreme was typical of strains of the fungus characterized in culture by broadly egg-shaped conidia of even size and shape (Fig. 27).

Medullary excipulum of textura intricata. Hyphae 1.5–5(8) μm in a gel.

The paraphyses were 1.0–2.0 μm thick. The tip was sometimes unswollen but usually more or less swollen, maximum diameter in different collections varying from 1.5 to 3.0 μm, rarely up to 4.0 μm. The tip was, however, not capitate.

The asci were 105–150 × 7–10 μm, always Melzer +.

Normal ascospores were 12–31 × 3.2–7.4 μm. The ascospores from the 16 separate collections varied as shown in Table II regarding mean of length and width, and minimum and maximum of length and width. The ascospores were usually pointed at each end and 0, 3, 5(7)-septate (Figs. 20, 21). Collections with ascospores having rounded ends were also found (Fig. 22). In several collections none of the spores had more than three septa.

In three collections quite abnormal ascospores (Fig. 23) were found besides normal-looking ones. Some of them were short, often with rounded ends and some were spherical, 6–7.5 μm in diameter. Somewhat angular spores were also found. Ascospores from two of the three collections did not germinate.

Small conidia or conidia-like cells in asci were always found in collections of ripe apothecia of *A. cylichnium* (Figs. 24, 25). These 'ascoconidia' were often abundant and conspicuous, but sometimes scarce and rather difficult to find. The 'ascoconidia' were undoubtedly often formed by abstriction from the ascospores, as depicted by Dennis (1956), but they might sometimes be primary, formed as indicated for *A. sarcoides*. The 'ascoconidia' in *A. cylichnium* were, however, of even shape and size (ca. 2–2.5 × 1.5–2.0 μm); in squash mounts many were seen as single conidia free from other cells.

Cultures. – Cultures were compared on malt agar. The colour was first white, later light to dark violet or greyish-violet in most cultures. Two isolates formed ripe apothecia on malt agar.

The hyphae were septate, 1–5 μm in diameter. Coils of hyphae were often found.

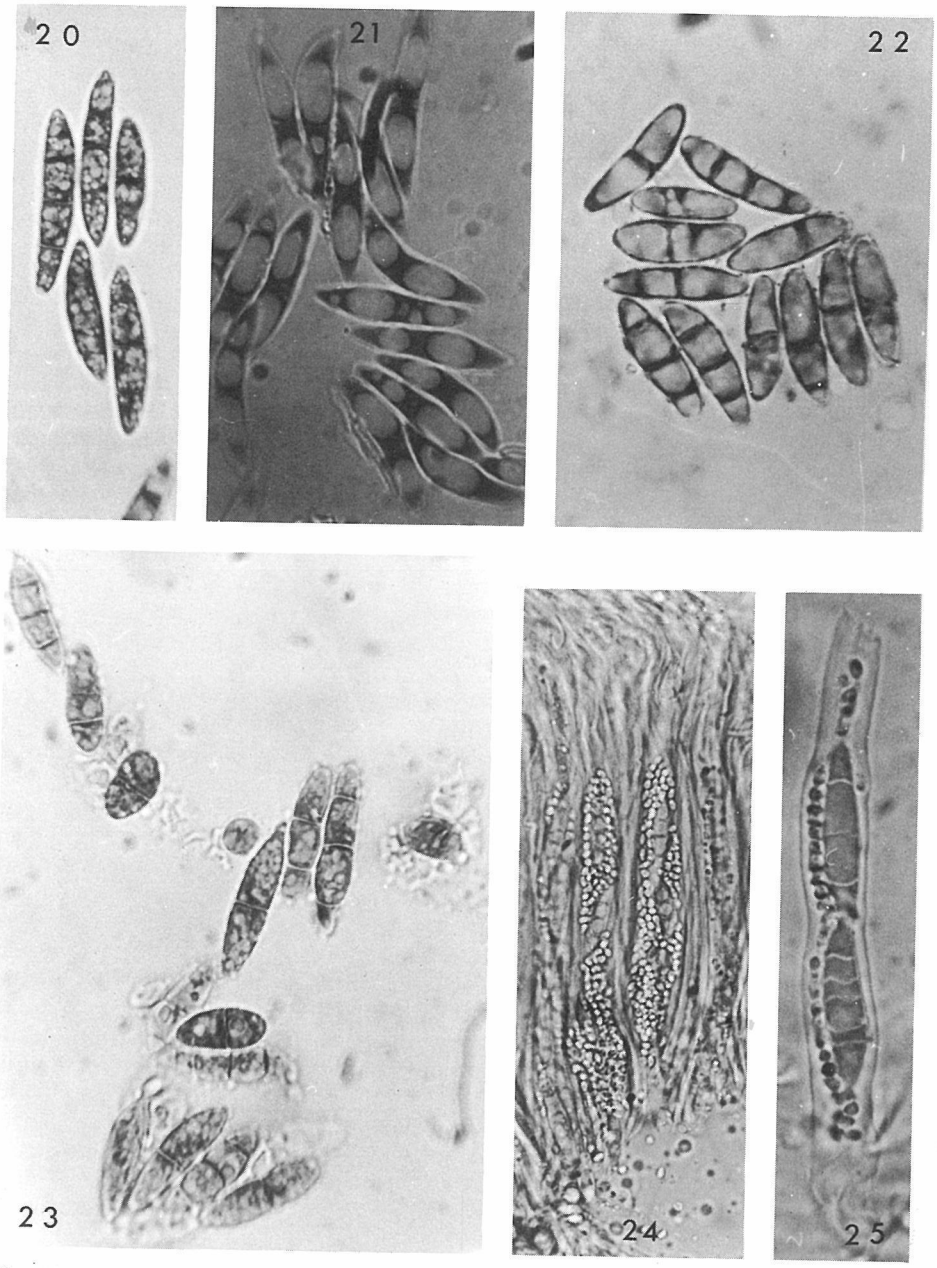
The conidiophores seldom grew out from hyphae coils. They were sometimes simple, unbranched, but usually branched, often *Penicillium*-like (Figs. 26–28). Very complex systems of conidiophores might be formed.

The conidia were hyaline and one-celled, extremely varying in shape. But in several cases only short, ovoid conidia, often with one big 'oil drop', were found (Figs. 26, 27); the size of these conidia was (2.0)2.3–4.0(5.0) × (1.3)1.5–2.8 μm; but for the separate isolates the variation might be much less – the conidia might be homogeneous as to shape and size. Young isolates from ascospores had in most cases only rather homogeneous, short, roundish conidia.

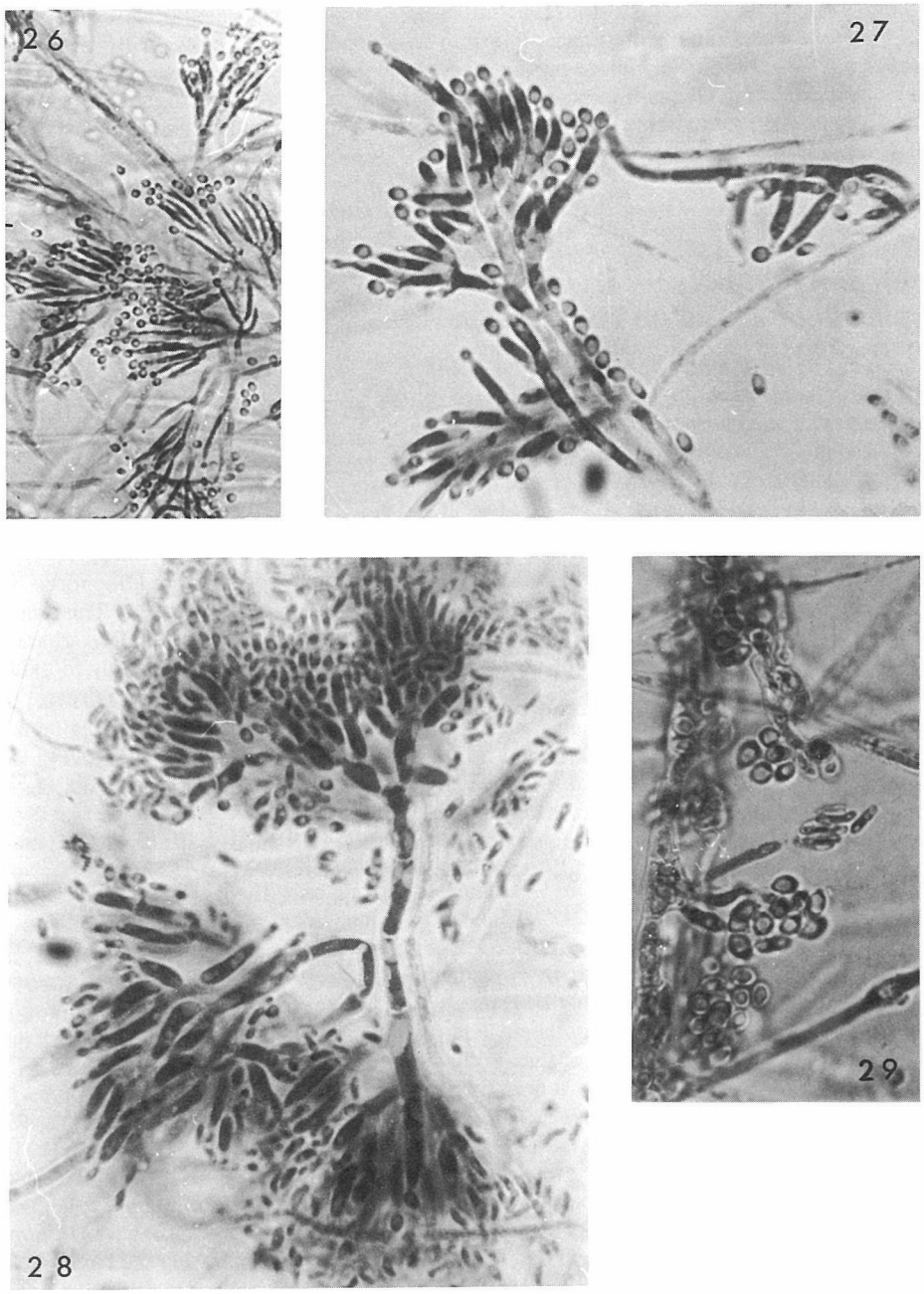
In many cases, however, relatively long and narrow, allantoid or rod-shaped conidia, 3.5–7.0 × 0.8–1.2(1.5) μm, were formed in addition to the shorter ones (Fig. 28); sometimes only such allantoid or rod-shaped conidia were found. Two examples are given below. From each of three fruitbodies from separate collections 10 monoascospore-isolates were made. In 29 of these isolates only the ordinary ovoid conidia were found. But in one isolate the form and size of the conidia were very variable, from broad, 3–5 × 2.0–2.6 μm, with one, sometimes two big 'oil drops', to narrow ones, 5–6 × 1.0–1.2 μm (Fig. 29). The last-mentioned isolate had a pure white, slow-growing mycelium with irregular contour, in contrast to the other, faster growing, more or less violet monoascospore-isolates. In another case a polyascospore-isolate was made from an apothecium with 0–7-septate ascospores 17.1–24.9 × 4.2–5.8 μm, often germinating in asci by roundish ascoconidia or by hyphae; but in culture on malt agar only narrow, allantoid conidia 4 × 1 μm, were found; ascospores in apothecia formed on malt agar by this isolate were 19–23 × 5–5.7 μm.

Cultures with the most homogeneous, broad, egg-shaped conidia (Fig. 27) were isolated from apothecia with the broadest and most indistinct transition from ectal to medullary excipulum (Fig. 19). There seemed to be a correlation between shape of conidia in culture and anatomy of excipulum.

The growth rate on malt agar for ascospore-isolates from 11 collections was 0.5–1.7 mm per 24 hours at 15 C.



Figs. 20–25. *Ascocoryne cylichnium*. Figs. 20–23. Ascospores thrown out from apothecia ×1,000. Fig. 20. Normal. Fig. 21. Sharply pointed. Fig. 22. Relatively broad with rounded ends. Fig. 23. Abnormal. Fig. 24. Asci with ascospores and ascoconidia. Hand section in Melzer's reagent ×500. – Fig. 25. Ascus with ascospores and ascoconidia. Squash slide in Melzer's reagent ×1,000.



Figs. 26–29. *Ascocoryne cylichnium*. Conidiophores and conidia from malt agar cultures. Mounted in lactophenol-cottonblue. Fig. 26. Ovoid conidia × 500. Fig. 27. Ovoid conidia in culture from the apothecium in Fig. 19 × 1,000. Fig. 28. Ovoid and allantoid conidia in culture from the apothecium in Fig. 18 × 1,000. Fig. 29. Monoascospore-isolate. Ovoid conidia, but allantoid conidia from one conidiophore × 1,000.

On gallic acid malt agar the reaction was lacking and the radial growth was 0–3 mm in seven days. On tannic acid malt agar the reaction was also lacking in all cases except one where it was weak (++); growth rate on this medium was 0–2 mm in seven days.

Comparison of *Ascocoryne sarcoides* with *Ascocoryne cylichnium* based on literature and own data

In the present investigation the anatomy of excipulum and the character of the ascoconidia or conidia-like cells in asci have been considered the two most reliable criteria discriminating between *A. cylichnium* and *A. sarcoides*. The collections have been divided by these criteria into the two species, which have then been described as to other characters elsewhere considered valuable for separation of the two species.

In the following we have compared the discriminating value of the different characters, using both earlier descriptions and our own observations.

Apothecia

Size and shape of the apothecia. – Some authors, for example Knapp (1924) and Dennis (1956), found that *A. cylichnium* produced wider apothecia than *A. sarcoides* did. In our collection it was quite the reverse (Table I). It must be concluded that width of the apothecia has no discriminating value. The shape of the apothecia may, however, on an average be more cupulate in *A. cylichnium* than in *A. sarcoides*.

Anatomy of excipulum. – Anatomy of excipulum and ascoconidia were the two characters which we used for separating our own material into the two species. The diagnostic value of these two characters is evident from the fact that we always got the same result independent of which of the two characters we used for identification. Further, there was a correlation between these two characters on the one side, and less reliable ones, such as shape of the paraphyses, size of ascospores, and shape of the conidia in culture on the other.

A. sarcoides was characterized by a distinct inner layer of ectal excipulum with roundish or somewhat angular, isodiametric or nearly isodiametric cells with abrupt transition to medullary excipulum. In *A. cylichnium* the inner layer of ectal excipulum had more or less of swollen cells; some of them might be isodiametric, but at the same time there were also more elongated cells, and the transition to medullary excipulum was even. There were transitions within *A. sarcoides* from the extreme *sarcoides*-type to more *cylichnium*-like types, and within *A. cylichnium* from types recalling *A. sarcoides* to types with no isodiametric cells.

It must be mentioned that our conception of the anatomy of the inner layer of ectal excipulum contrasts sharply with the figure of ectal and medullary excipulum in *A. cylichnium* given by Dennis (1956, Fig. 151); in our opinion Dennis depicted a typical *A. sarcoides*, but he did not discuss this character.

Occasionally the outer layer of ectal excipulum formed conidiophores abstricting allantoid conidia (Fig. 4). This was apparently a rather reliable specific character for *A. sarcoides*, but it was rarely found.

Paraphyses. – Paraphyses which were strongly swollen and capitate at the tip were found only in *A. sarcoides*, and paraphyses not at all swollen at the tip only in *A. cylichnium*. Apothecia where none of the paraphyses were capitate, but more or less swollen at the tips were, however, common in both species. Therefore only the extremes, capitate or completely unswollen paraphyses, had diagnostic value for separating the two species.

Asci. – Generally the asci seemed to be bigger in *A. cylichnium* than in *A. sarcoides* (Tab. I). In many cases size of asci was of little value for separating the species.

Ascospores. – The ascospores have been reported to be smaller in *A. sarcoides* than in *A. cylichnium*, e.g. by Knapp (1924) and Dennis (1956). We found less difference than reported by them (Tab. I). In some collections studied by us the size of the spores was intermediary and of no use for separating the two species (Tab. II).

Generally *A. sarcoides* has been reported to have ascospores with rounded ends and *A. cylichnium* spores with acute ends. But the shape of the spores was very variable. In some collections of *A. sarcoides* many of the spores had rather acute ends, and in some collections of *A. cylichnium* the spores had rounded ends. In both species apothecia with both normal-looking and abnormal ascospores were found, often

Table I. Size of apothecia, asci, and ascospores given by various authors

	A. sarcoides	*A. cylichnium*
Diameter of apothecia up to (mm):		
KNAPP (1924)	13	25
DENNIS (1956)	10	15
Our measures	23	12
Asci (µm):		
DENNIS (1956)	90-120 × 8-10	140-200 × 10-12
Our measures	85-130 × 6.5-10	105-150 × 7-12
Ascospores (µm):		
KNAPP (1924)	12-18 × 4-5	23-30 × 6-7
DENNIS (1956)	10-15(19) × 3-5	(14)18-30 × 4-6
Our measures	10-24 × 3.4-6.1	12-31 × 3.2-7.4

within one ascus. It was evident that in many cases size and form of the ascospores were of no use for separating the two species.

Ascoconidia. – Ascoconidia have been said to be lacking in *A. sarcoides*, and to be characteristic of *A. cylichnium*. Knapp (1924), however, reported egg-shaped conidia also in the asci of *A. sarcoides*. We found chains of small roundish or elongated cells in the asci in two of the collections of *A. sarcoides*. But these small cells were not so even as to size and form as the characteristic ascoconidia found in *A. cylichnium*, and they did not easily separate. In fact, besides the anatomy of excipulum, we regard lack of the characteristic ascoconidia in *A. sarcoides* as the best character separating it from *A. cylichnium*. In *A. cylichnium* they have always been found in ripe apothecia, at least in a few of the asci (cf. p. 201). The absence or presence of such ascoconidia was well correlated with other, less reliable characters such as shape of the paraphyses, size of the ascospores, and shape of the conidia in culture.

Cultures

No significant difference was found between cultures of *A. sarcoides* and *A. cylichnium* as to colour and growth rate on malt agar or as to appearance and size of the hyphae.

Table II. Variation in size of ascospores among separate collections

Ascospores	*A. sarcoides* µm	*A. cylichnium* µm
Total in all collections	10.1-23.9 × 3.4-6.1	12.0-31.0 × 3.2-7.4
Mean in the separate collections	12.8-19.9 × 4.2-5.6	18.7-25.8 × 4.0-6.4
Minimum in the separate collections	10.1-15.8 × 3.4-5.1	12.0-24.0 × 3.2-5.7
Maximum in the separate collections	14.0-23.9 × 4.5-6.1	20.1-31.0 × 5.0-7.4

Table III. Size of conidia in culture on malt agar at ca. 20 C

Shape of conidia	*A. sarcoides* µm	*A. cylichnium* µm
Allantoid or rod-shaped	3.0-6.0(7.5) × 0.8-1.4(1.9)	3.5-7.0 × 0.8-1.2(1.5)
Ovoid	2.0-3.0 × (0.8)1.2-1.6	(2.0)2.3-4.0(5.0) × (1.3)1.5-2.8

Allantoid or rod-shaped conidia, 3.0–6.0(7.5) × 0.8–1.4(1.9) µm, were characteristic of *A. sarcoides,* but also shorter, more roundish conidia 2.0–3.0 × (0.3)1.2–1.6 µm were found. Sometimes *A. cylichnium* formed allantoid or rod-shaped conidia similar to those of *A. sarcoides;* occasionally only such conidia were found, but more often they occurred together with shorter, more roundish conidia (Tab. III, Figs. 28, 29). Identification of such cultures seemed often impossible. But only cultures from *A. cylichnium* were found to form exclusively rather uniform, broad, ovoid conidia (Fig. 27). Positive reaction on tannic acid malt agar may indicate *A. sarcoides,* negative reaction *A. cylichnium.*

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