

TWO RARE COPROPHILOUS ASCOMYCETES FROM NORWAY

J. VAN BRUMMELEN¹ & R. KRISTIANSSEN²

Two uncommon coprophilous ascomycetes, *Ascobolus cervinus* and *Caccobius minusculus*, only recorded once, each from two different continents, are reported for the first time from Norway. Both are provided with new descriptions and compared with authentic material.

During the last few years, the second author has found a number of rare or unusual coprophilous discomycetes new to the mycoflora of Norway, such as *Pseudascozonus racemosporus* Brumm. (Kristiansen & Schumacher, 1993), *Coprotus breviascus* (Velen.) Kimbr. et al., *Ascobolus degluptus* Brumm., *Saccobolus citrinus* Boud. & Torrend (Kristiansen, 1993), *Ascodesmis nana* Brumm., *Ascodesmis nigricans* Tiegh., *Ascodesmis sphaerosporus* Obrist (Kristiansen, 1994), and *Pseudombrophila virginea* (Svrček & Moravec) Brumm. (van Brummelen, 1995).

Now two very uncommon species of coprophilous ascomycetes, *Ascobolus cervinus* Berk. & Broome and *Caccobius minusculus* Kimbr. in Kimbr. & Korf, have been collected from Norway. Both species were only known from their type localities in Sri Lanka (Ceylon) and Canada, respectively. Full descriptions, based on recent findings in Norway and study of authentic material, are provided.

Ascobolus cervinus — Figs. 1, 2

Ascobolus cervinus Berk. & Broome, J. Linn. Soc. Lond. (Bot.) 15 (1876) 85.

Holotype: Sri Lanka (Ceylon), Peradeniya, on dung of deer (?), II.1869, *Thwaites 1122* (K).

Illustration. J.-O. Aanæs, *Blekksoppen* 24 (69) (1996) 22. 1996 (rather old fruit-bodies).

Ascomata apothecial, gregarious or crowded, superficial, sessile, on a broad base, up to 2.5 mm across and 1 mm high. Receptacle at first closed and subglobular, then opening at the top and hemispherical, finally expanding and becoming scutellate, often irregularly shaped by mutual pressure, brownish olive-green, rather dark at maturity, fleshy, finely verrucose or furfuraceous, with a prominent margin. Disc at first concave to flat, finally often slightly convex, roughened by the protruding tips of ripe asci, at first yellow-green to pale olive-green, then dark olive, becoming dark brown to almost black at maturity. Hymenium about 190 µm thick. Hypothecium clearly differentiated, 28–35 µm thick, of isodiametric thin-walled cells 5–11 µm wide. Medullary excipulum 160–250 µm thick, hyaline, consisting of fairly thin-walled isodiametric to oblong cells, 7–45 × 5–20 µm and a few subcylindrical hyphae about 4–5 µm wide (textura globulosa). Cortical excipulum near the base 35–150 µm thick, at the margin 18–40 µm wide, with brownish to dark brown amorphous intercellular pigment, consisting of closely compacted thick-walled subglobular cells 5–45 × 5–35 µm (textura globulosa), over the whole surface with increasing deposits of

1) Rijksherbarium / Hortus Botanicus, P.O. Box 9514, 2300 RA Leiden, The Netherlands.

2) P.O. Box 32, N-1650 Sellevakk, Norway.

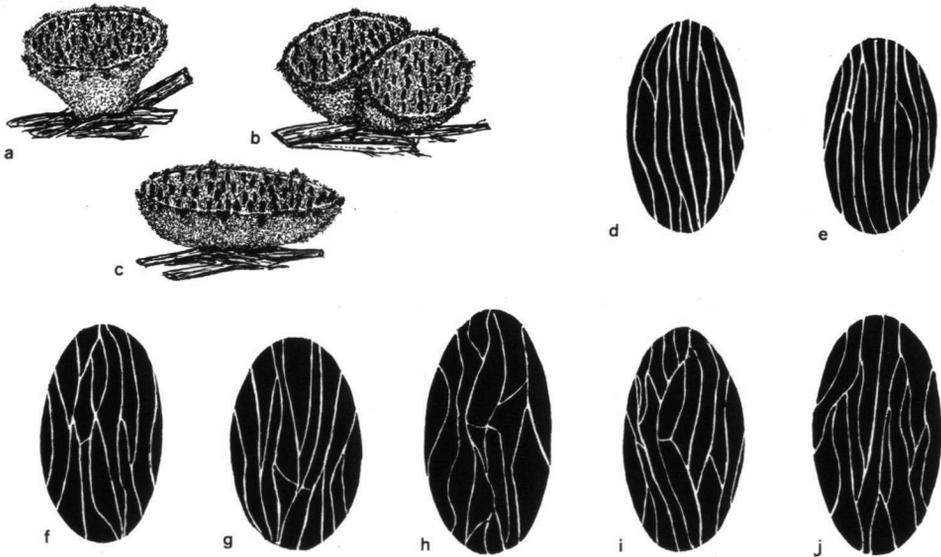


Fig. 1. *Ascobolus cervinus*. — a–c. Habit of fruit-bodies, $\times 16$; d–j. ascospores, $\times 1600$ (a–c, f–j, from *R. Kristiansen RK 96.18*; d, e, from holotype).

granular and amorphous brown pigment, covered with small, often rather sparse, irregular groups of isodiametric and oblong strongly pigmented cells $5\text{--}15 \times 4\text{--}9 \mu\text{m}$. Asci cylindrical, gradually narrower towards the base, rounded above, $165\text{--}185 \times 14\text{--}17 \mu\text{m}$, 8-spored, the wall staining faintly blue with iodine. Ascospores 1–2-seriate, ellipsoid (length-width ratio (Q) 1.7–1.9, average 1.83), at first hyaline, then purplish, becoming purplish brown at maturity, $(14.1\text{--})15.4\text{--}16.0\text{--}(17.7) \times (7.4\text{--})8.0\text{--}9.0\text{--}(9.6) \mu\text{m}$ (ornamentation included), without oil-globules or granules, ornamented with a pattern of more or less longitudinal anastomosing fine lines (6–8 visible at lateral view). Paraphyses frequent, septate, cylindrical, branched, hyaline, $2.0\text{--}2.5 \mu\text{m}$ thick, not or scarcely enlarged up to $2\text{--}4 \mu\text{m}$ at the tip, embedded in yellowish green to olive brown mucus strongly darkening with age.

On dung of elk (*Alces alces*) and probably of deer.

Specimens examined. NORWAY: Buskerud, Øvre Eiker, Røkkebergtjern, c. 200 m alt., on elk dung in spruce wood, 4.VI.1995, *R. Kristiansen RK 95.62* (L); Hedmark, Sør-Odal, Galterud, Molykkja, c. 300 m alt., on elk dung in spruce wood, 25.V.1996, *A. Sagbakken & R. Kristiansen RK 96.13* (L); idem, 23.VI.1996, *RK 96.18* (L); Hedmark, Kongsvinger, Serkilampi nature reserve, c. 300 m alt., on elk dung in spruce wood, 24.VI.1996, *R. Kristiansen RK 96.19* (L); Hordaland, Voss, on elk dung, IV.1976, *I. Trøen 57b* (BG); Finnmark, Sør-Varanger, Øvre Pasvik National Park, on elk dung, 13.VII.1968, *S. Sivertsen* (TRH); Oppland, Vågarn, Glitteheim-road, on elk dung, 27.VII.1998, *R. Kristiansen RK 98.76* (L). — SRI LANKA: Peradeniya, on dung of deer (?), II.1869, *Thwaites 1122* (holotype of *Ascobolus cervinus* Berk. & Broome; K).

For a long time, *Ascobolus cervinus* was only known from Berkeley & Broome's original short description and later from the more complete redescription, based on the rather sparse and not yet fully mature type material, by van Brummelen (1967).

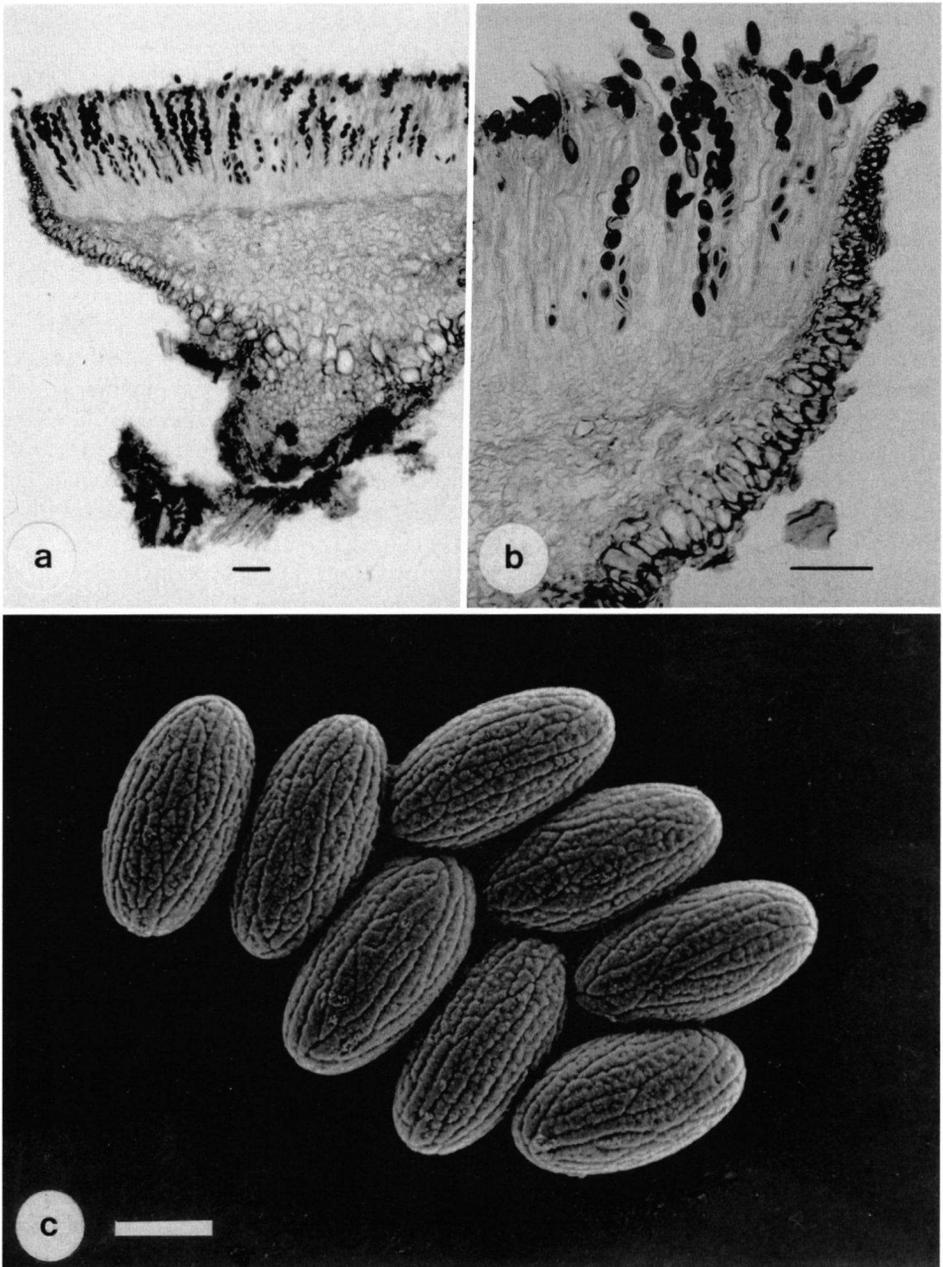


Fig. 2. *Ascobolus cervinus*. — a. Photomicrograph of part of median section through ripe fruit-body (bar = 50 µm); b. idem, detail near margin (bar = 50 µm); c. scanning electron micrograph of ascospores (bar = 5 µm) (all from R. Kristiansen RK 96.18).

Especially after the study of some rich collections (*R. Kristiansen RK 96.18* and *RK 96.19*) from the district of Hedmark in Norway, *A. cervinus* can be identified with certainty, although investigation of several earlier Norwegian collections from the herbaria of Trondheim (TRH) and Bergen (BG) proved them to belong also to this species.

In a thesis on Norwegian coprophilous discomycetes, Aas (1978: 21) described an '*Ascobolus* sp.' based on a collection from elk dung from the district of Hordaland (*Troen 57b*), which proves to belong to this species.

In Norway, this species has only been found on dung of elk, both in the southern and northern parts of the country. It is remarkable that it has not been found in the south-eastern coastal areas, despite elk being very common there and the frequent examination of elk dung. Based on the Norwegian records, the species seems to have a vernal appearance, April to July, depending on the latitude; which also proves that it tolerates a cold climate. Despite intense search, it has not been found anywhere during summer or autumn.

Recently, *Ascobolus cervinus* has been reported from the Shaanxi province of China (Zhuang, 1996). But a study of the description and the material concerned (*Q.-M. Ma et al. 2485*, HMAS 33705), revealed that the mature ascospores are wholly hyaline and do not possess the violet or purplish pigment layer, characteristic of the genus *Ascobolus* Pers. The longitudinal ridges of the ascospore ornamentation easily stain with methyl blue. This fungus, which is probably not coprophilous but growing on vegetable debris, proved to be identical with *Peziza urinophila* Y.-Z. Wang & Sagara, described from Taiwan and Japan from vegetable debris and forest litter after application of urea or after decomposition of animal matter (Wang & Sagara, 1997).

By this more extensive study, it becomes easier to distinguish *Ascobolus cervinus* from some related species, such as *A. crenulatus* P. Karst., *A. michaudii* Boud., *A. castorensis* Aas, and *A. fushanus* Y.-Z. Wang & Brumm. A macroscopic distinctive feature of *A. cervinus* is the absence of yellowish or yellowish green pigments, present in the receptacle and disc of the other four species. On the contrary, *A. cervinus* becomes dark brown by the presence of considerable amounts of an olive green to dark olive brown amorphous pigment; moreover, the surface of the receptacle is the least warty or furfuraceous among these species.

Apart from other characters, there are microscopically mostly clear differences in the shape and size of the ascospores. As compared with *A. cervinus*, the ascospores in *A. fushanus* (Wang & van Brummelen, 1997) have only a few longitudinal striae and are of about the same length, but more narrowly ellipsoid in shape ($14-18 \times 6.5-8 \mu\text{m}$; $Q = 2.01-2.36$). In *A. michaudii* the ascospores are considerably larger ($17-22 \times 9.5-12 \mu\text{m}$), while in *A. crenulatus*, the ascospores are clearly smaller and more broadly ellipsoid ($11.1-12.9 \times 6.1-7.6 \mu\text{m}$; $Q = 1.53-1.72$). *Ascobolus castorensis*, which seems to be restricted to dung of beaver (*Castor fiber*), has ascospores of about the same size as *A. cervinus*, but is well-characterized by the presence of many prominent yellow-orange warts on the upper part of the receptacle (Aas, 1977).

Caccobius minusculus — Fig. 3

Caccobius minusculus Kimbr. in Kimbr. & Korf, *Am. J. Bot.* 54 (1967) 22, figs. 4a-f. — Type: South of Whitney, Nipissing Distr., Ontario, Canada, on rabbit dung, 26.IX.1956, *R.F. Cain* (holotype CUP 47615; isotype TRTC 32390).

Ascomata apothecial, solitary or in small groups, superficial, sessile, 0.12–0.20 mm diam., 0.18–0.25 mm high. Receptacle cylindrical to obconical, sometimes becoming pulvinate,

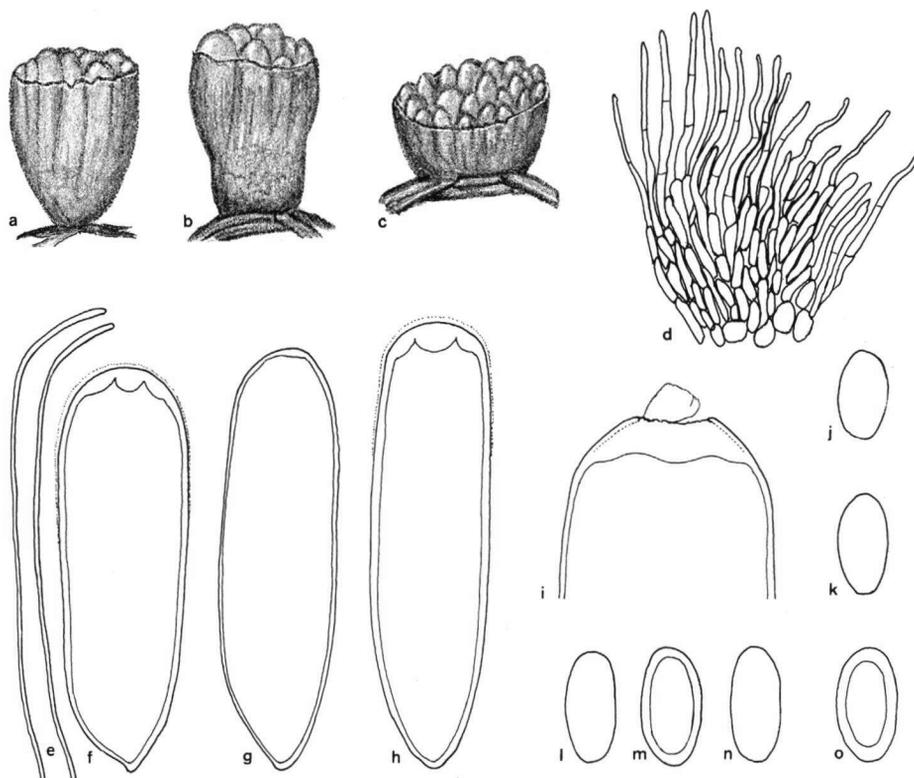


Fig. 3. *Caccobius minusculus*. — a–c. Habit of fruit-bodies, $\times 90$; d. detail of excipulum seen from outside, $\times 250$; e. paraphyses, $\times 500$; f–h. asci, $\times 500$; f, h, in 10% NH_4OH , g in water; i. detail of top of dehiscent ascus, $\times 800$; j–o. ascospores, $\times 2700$ (all from R. Kristiansen & A. Sagbakken RK 96.130).

pinkish red; margin not differentiated. Disc flat to convex, roughened by the prominent tips of asci, pinkish red. Hymenium about $150\ \mu\text{m}$ thick. Hypothecium scarcely differentiated. Excipulum not clearly differentiated in a cortex and a medulla, near the base $50\text{--}70\ \mu\text{m}$ thick of isodiametric cells $5\text{--}9\ \mu\text{m}$ diam. (textura globulo-angularis), only a single layer of rows of hyaline oblong cells at the sides, $10\text{--}16 \times 2.5\text{--}5.0\ \mu\text{m}$ (textura porrecta), smooth. Asci broadly cylindrical with a short stalk, rounded above, the ascus wall strongly swelling or even dissolving in 10% NH_4OH , $(80\text{--})130\text{--}150 \times 33\text{--}36\ \mu\text{m}$, about 1000-spored, with the apex strongly thickened on the inner side ($2.5\text{--}5.0\ \mu\text{m}$), not blue with iodine. Ascospores ellipsoid (length-width ratio 2.0–2.8, average 2.2), hyaline, $(4.5\text{--})5.4\text{--}6.0 \times 2.3\text{--}2.8\ \mu\text{m}$, without oil globules, air bubbles, or granules, smooth. Paraphyses frequent, septate, cylindrical, simple, hyaline to pale pinkish, $1.8\text{--}2.5\ \mu\text{m}$ thick, not or slightly enlarged up to $3.5\ \mu\text{m}$ at the tip, not embedded in mucus.

On rather old rabbit dung.

Specimens examined. NORWAY: Østfold, Hvaler, N. Kirkøy, Utengen, on rabbit dung, 16.XI.1996 and 5.XII.1996, R. Kristiansen & A. Sagbakken RK 96.130 (L); id. 18.X.1997, R. Kristiansen RK 97.20 (L). — CANADA: Ontario, Nipissing Distr., South of Whitney, on rabbit dung, 26.IX.1956, R.F. Cain (isotype of *Caccobius minusculus* Kimbr. in Kimbr. & Korf; TRTC 32390).

Caccobius minusculus was described from a specimen in Dr. R.F. Cain's herbarium, collected in 1956 in Ontario, Canada (Kimbrough & Korf, 1967). The new genus *Caccobius* Kimbr. in Kimbr. & Korf was created 'for species intermediate between *Ascozonus* and *Thelebolus*'.

The species is presumably psychrophilic or strongly tolerant of cold growing conditions, since it was found after a long period of cold weather and snow. The Norwegian locality is situated on a group of islands at the south-eastern outlet of the Oslofjord, close to the Swedish border. The locality is an accessible point on acidic rock. The sparse vegetation consists mostly of small pines (*Pinus sylvestris*) and carpets of heather (*Calluna vulgaris*) with some shallow marshes. The place is frequented by deer and elk, judging from the amounts of dung of these animals. Other coprophilous species recorded simultaneously on these substrates are *Ascozonus woolhopensis* (Berk. & Br. in Renny) Boud., *Thelebolus polysporus* (P. Karst.) Otani & Kanzawa, *Thelebolus stercoreus* Tode: Fr., *Trichobolus sphaerosporus* Kimbr. in Kimbr. & Korf, *Ascobolus brassicae* Crouan, and *Ascobolus sacchariferus* Brumm.

The pinkish red colour of the fresh fruit-bodies, already observed in the field, is a constant character, which may gradually fade in dried material. The Canadian material was described as 'pallid to white'.

The 'mucilaginous sheath around each ascus', as described by Kimbrough (Kimbrough & Korf, 1967), is certainly due to the presence of a very thick periascus (van Brummelen, 1998). The paraphyses are long and often overarch the ascus top, but in both collections of *Caccobius minusculus* studied, no evidence was found for the presence of an epithecium or 'pseudoeicipulum above the true excipulum' as described by Kimbrough (l.c.).

Caccobius was considered to be related to non-operculate genera of the Thelebolaceae (Korf, 1972; Kimbrough, 1972) or even to the 'inoperculates', because of the presence of a 'plug' in the top of the ascus, staining with Waterman's blue-black ink (Samuelson, 1978; Samuelson & Kimbrough, 1978).

A more detailed study of the ascus structure in the genera of the Thelebolaceae (van Brummelen, 1998) revealed a wide variation in structure and even more in the function of the opening mechanism of the ascus.

Large amounts of inner wall material form a central thickening at the top of the ascus. Usually the thickening is central, but it may also be located more towards the side of the apex. In *Caccobius*, as well as in *Ramgea*, *Pseudascozonus*, and some species of *Thelebolus*, opening of the ascus occurs after splitting within the inner wall layer in the apex more or less parallel to the ascus surface. This is considered the typical *Thelebolus*-type of ascus structure (van Brummelen, 1998). The central thickening in *Caccobius* may reach a thickness of 3 µm and a width of 4–6 µm and is sharply delimited by sharp folds in the inner wall layer in the subapical region. At first, this thickening shows little or no differentiation. Later it becomes clearly stratified by strata of alternating low and strong reactivity after the Thiéry-test. On still further ripening, the stratification becomes less evident, but can still be seen as fine horizontal lines. When treated with Waterman's blue-black ink, the irregular boundary planes of the cavities and the fine lines within the central thickening especially stain blue.

In a recent phylogenetic analysis of 21 selected species of Ascomycotina with DNA sequencing techniques (Landvik et al., 1998), three species of Thelebolaceae (*Thelebolus stercoreus* Tode: Fr., *Ascozonus woolhopensis* (Berk. & Br. in Renny) Boud., and *Caccobius minusculus*) were included. The results show, that these Thelebolaceae group together and

indicate a closer phylogenetic relationship with representatives of the inoperculate discomycetes, like *Leotia lubrica* Pers. and *Microglossum viride* (Pers.) Gillet, than with members of the operculate Pezizales. This confirms earlier sequencing studies by Momol & Kimbrough (1994), who found that an unidentified species of *Thelebolus* was not related to seven members of operculate Pezizales. With their methods, Landvik et al. (1998) were not able to resolve the phylogenetic relationships within this group of three Thelebolaceae.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. Sara Landvik, of the Institute of Biology, University of Oslo, for the preparation of the scanning electron micrograph of ascospores of *Ascobolus cervinus*. We also thank the curators of the herbaria of Trondheim (TRH), Bergen (BG), Toronto (TRTC), and Beijing (HMAS) for the loan of herbarium specimens under their care. We are indebted to Miss Aud Sagbakken, Kongvinger, Norway, for placing a rich collection of *Ascobolus cervinus* at our disposal. Thanks are due to Mr. J. T. Palmer, Sutton Weaver (UK) for linguistic advice.

REFERENCES

- Aas, O. 1977. *Ascobolus castorensis* n. sp. on dung of beaver in Norway. *Norw. J. Bot.* 24: 57–58.
- Aas, O. 1978. *Kopprofile discomycetar (Ascomycetes: Discomycetes Operculati = Pezizales) i Noreg*. Unpublished Thesis Univ. Bergen.
- Brummelen, J. van. 1967. A world-monograph of the genera *Ascobolus* and *Saccobolus* (Ascomycetes, Pezizales). *Persoonia Suppl.* 1.
- Brummelen, J. van. 1995. A world-monograph of the genus *Pseudombrophila* (Pezizales, Ascomycotina). *Libri Botanici* 14: 1–117.
- Brummelen, J. van. 1998. Reconsideration of relationships within the Thelebolaceae based on ascus ultrastructure. *Persoonia* 16: 425–469.
- Kimbrough, J.W. 1972. Ascus structure, ascocarp ontogeny, and a natural classification of the Thelebolaceae. *Persoonia* 6: 395–404.
- Kimbrough, J.W. & R.P. Korf. 1967. A synopsis of the genera and species of the tribe Thelebolaceae (= Pseudoascoboleae). *Am. J. Bot.* 54: 9–23.
- Korf, R.P. 1972. Synoptic key to the genera of the Pezizales. *Mycologia* 64: 937–994.
- Kristiansen, R. 1993. Møkk - et spennende substrat for begersopper. *Agarica* 12 (21): 122–137.
- Kristiansen, R. 1994. *Ascodesmis* (Pezizales) i Norge, en sjelden koprofil slekt eller bare oversett? *Agarica* 13 (22): 87–100.
- Kristiansen, R. & T. Schumacher. 1993. Nye operkulerte begersopper i Norges flora. *Blyttia* 51: 131–140.
- Landvik, S., R. Kristiansen & T. Schumacher. 1998. Phylogenetic and structural studies in the Thelebolaceae (Ascomycota). *Mycoscience* 39: 49–56.
- Momol, E.A. & J.W. Kimbrough. 1994. Phylogenetic analysis of selected genera of Pezizales, inferred from 5.8S rDNA, ITS1 and ITS2 sequences. *Syst. Ascom.* 14: 1–12.
- Samuelson, D.A. 1978. Asci of the Pezizales. VI. The apical apparatus of *Morchella esculenta*, *Helvella crispa*, and *Rhizina undulata*. General discussion. *Can. J. Bot.* 56: 3069–3082.
- Samuelson, D.A. & J.W. Kimbrough. 1978. Asci of the Pezizales. IV. The apical apparatus of *Thelebolus*. *Bot. Gaz.* 139: 346–361.
- Wang, Y.-Z. & J. van Brummelen. 1997. A new species of *Ascobolus* from Taiwan. *Mycotaxon* 65: 433–446.
- Wang, Y.-Z. & N. Sagara. 1997. *Peziza urinophila*, a new ammonophilic discomycete. *Mycotaxon* 65: 447–452.
- Zhuang, W.-Y. 1996. Some new species and new records of discomycetes in China. VI. *Mycotaxon* 59: 337–342.