

Helotium separabile - a species of *Phialina* on *Rubus*

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Abstract: A lectotype for *Helotium separabile* P. KARST. is designated and the delimitation of the species clarified. The presence of tapering hairs with filiform apices and yellow pigment is stressed as a taxonomic character and the species is transferred to the genus *Phialina* HÖHN. Variability is mostly present in the thickness of gelatinization of hyphal walls in the excipulum. The species is also confirmed to occur in North America. It is apparently confined to stems of *Rubus* spp. *Helotium firmulum* P. KARST. is shown to be a synonym.

Zusammenfassung: Ein Lectotyp für *Helotium separabile* P. KARST. wird designiert und die Abgrenzung der Art geklärt. Die Haare, welche in eine kurzfädige Spitze zusammengezogen sind, und das gelbe Pigment werden als taxonomisch entscheidende Merkmale angesehen; die Art wird daher in die Gattung *Phialina* HÖHN. gestellt. Variabel sind die untersuchten Populationen vor allem im Ausmaß der gelatinösen Verdickung der Hyphenwände im Excipulum. Zahlreiche Proben aus Nord-, West- und Mitteleuropa sowie eine aus dem temperaten östlichen Nordamerika konnten untersucht werden. Die Art wächst anscheinend ausschließlich auf abgestorbenen Sprossen von *Rubus*-Arten. *Helotium firmulum* P. KARST. ist ein Synonym.

In 1871 KARSTEN described a new *Helotium* from stems of *Rubus idaeus* L. from Mustiala, Finland. He characterized the species by the following diagnosis (here translated from the Latin):

"Apothecia growing densely together to gregarious, sessile, first shortly downy, then becoming glabrous, flattened, white to whitish, disc yellowish, margin entire, soon flexuous, diam. 0.5-1 mm. Asci cylindric-clavate, apex not stained in iodine, 60-80 µm long, 5-6 µm wide. Spores fusoid-elongate, two-guttulate to falsely one-septate with thin septum, straight to curved, 8-15 µm long, 2-3 µm wide. Paraphyses gracile. On dry stems of *Rubus idaeus* near Mustiala, observed several times in July - October."

In his revision KARSTEN (1885) retained the species in *Helotium*, amongst the taxa classified together as having sessile and soft apothecia and typically guttulate spores, rarely aguttulate or falsely septate spores.

The first modern treatment of the species was published by DENNIS (1956). DENNIS had not seen any authentic material, but he was able to link KARSTEN's diag-

nosis to the correct taxon. Three of the four collections cited by him are clearly conspecific and concur with the original material. All these were growing on *Rubus idaeus*. The fourth, collected on *Rubus fruticosus*, represents an interesting, unnamed, hairy taxon with quite different spores.

DENNIS (1956) noted the presence of *Phialina*-type hairs on the excipulum but classified the species first in *Helotium* and a few years later in *Hymenoscyphus* (DENNIS 1964). This placement has persisted up to recent years (e.g., HOLM & NANNFELDT (†) 1990, CANNON & al. 1985, ELLIS & ELLIS 1985). The species was omitted from a synopsis of *Phialina* by HUHTINEN (1990). Recently KARSTEN's species has been included in *Calycina* NEES ex GRAY (WEBER 1992, KRIEGLSTEINER 1993) but no new combination has been proposed.

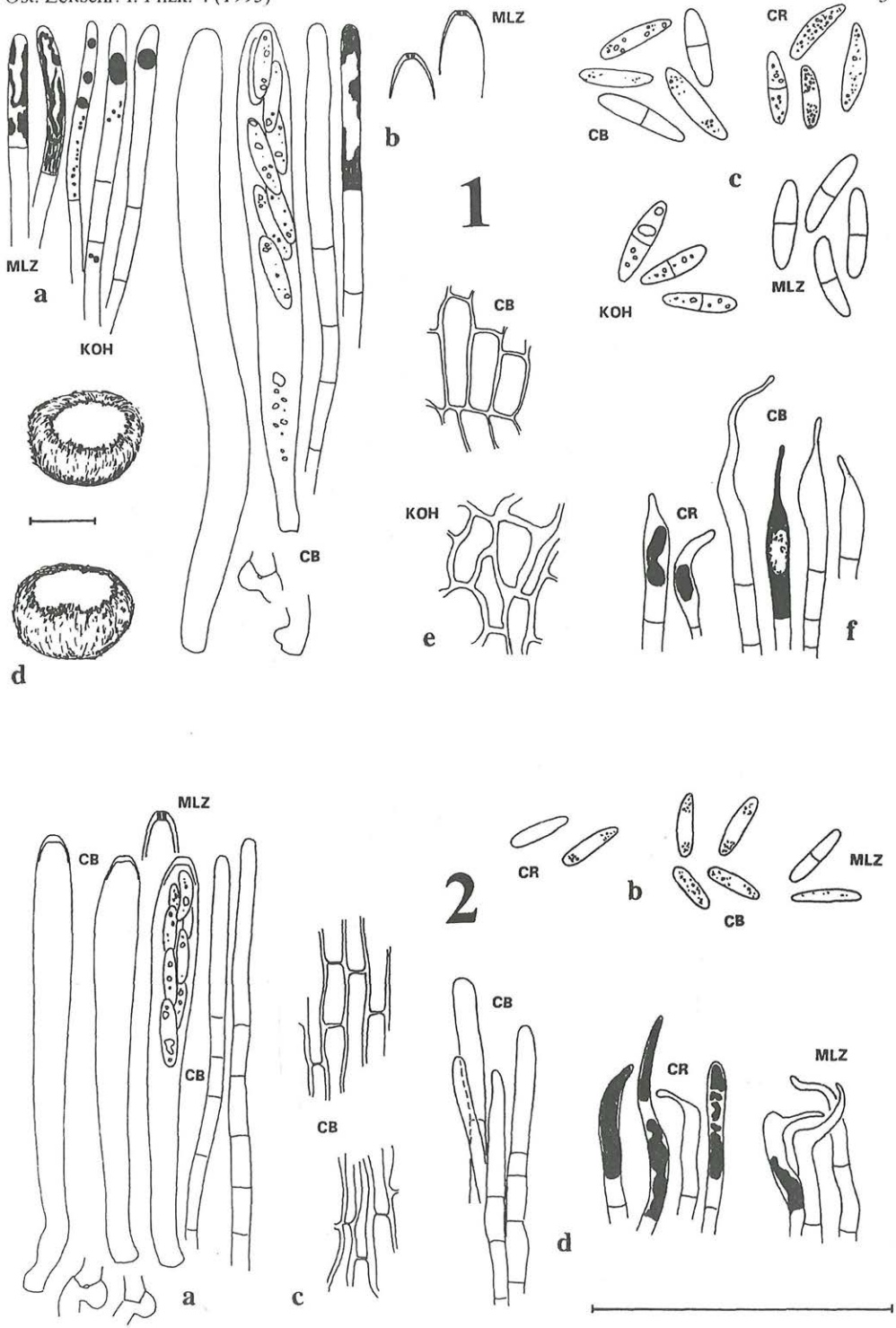
The present treatise is the first to include original material. No material was found in KARSTEN's herbarium (H), but one collection exists in S, apparently sent by KARSTEN to STARBÄCK. Although the specimen is not annotated by KARSTEN, it can be regarded rather safely as part of the cited type material: The date of collection and the ecology concur with the protologue. Because more than a single collection were cited indirectly by KARSTEN, the specimen cannot be regarded as the holotype or an isotype, but it agrees with the original diagnosis to such an extent that it can well be designated as the lectotype of *Helotium separabile* P. KARST.

KARSTEN's original diagnosis differs from the present material in two respects. (1) He stated the asci to be non-staining in iodine. This is most likely an erroneous observation because such a feature was not observed in any of the collections studied, not even in KARSTEN's material. Only GRELET (1954) concurs that there was no reaction in iodine. Unfortunately, the material on *Rubus idaeus* from France cited by him could not be examined for the present study. (2) KARSTEN's use of the term "graciles" concerning the paraphyses is misleading. In *Helotium* he used "filiformes" for paraphyses which were between 1.5-2.0 µm thick, according to his measurements. For the paraphyses in, for example, *Helotium parile* P. KARST. and *H. lutisedum* (P. KARST.) P. KARST. he also used the term "graciles". These taxa have paraphyses ranging from 1.5-3.0 µm in width. Hence, KARSTEN's terminology is not necessarily in conflict with our lectotype material.

***Phialina separabilis* (P. KARST.) HUHTINEN & SCHEUER, comb. nova**

Basionym: *Helotium separabile* P. KARST., Bidrag Kännedom Finlands Natur Folk **19**: 118, 1871. - *Pseudohelotium separabile* (P. KARST.) SACC., Syll. Fung. **8**: 297, 1889. - *Pezizella separabilis* (P. KARST.) REHM ex K. KRIEG., Fungi Saxonici 1239, 1897. - *Belonium separabile* (P. KARST.) REHM, Ascomyceten 1217, 1898. - *Mollisiella separabilis* (P. KARST.) BOUD., Histoire et classification des discomycètes d'Europe, p. 142, 1907. - *Hymenoscyphus separabilis* (P. KARST.) DENNIS, Persoonia **3**: 77, 1964.

Lectotype (designated here): [Finland, Tavastia australis, Tammela,] Mustiala, [on dry stems of *Rubus idaeus*], IX. 1866, KARSTEN (S).



Figs. 1-2. *Phialina separabilis*. - 1. Lectotype of *Helotium separabile*: (a) paraphyses (b) asci and paraphyses (c) ascospores (d) dried apothecia (e) ectal excipulum in surface view (f) marginal hairs. - 2. Lectotype of *Helotium firmulum*: (a) asci and paraphyses (b) ascospores (c) ectal excipulum in surface view (d) marginal hairs. - Bar: 50 µm, for apothecia 100 µm.

Synonym: *Helotium firmulum* P. KARST., Meddeland. Soc. Fauna Fl. Fenn. **16**: 33, 1888. - *Pseudohelotium firmulum* (P. KARST.) SACC., Syll. Fung. **8**: 292, 1889. - *Tapesia firmula* (P. KARST.) BOUD., Histoire et classification des discomycètes d'Europe, p. 139, 1907.

Lectotype (designated here): [Finland, Tavastia australis, Tammela,] Mustiala, ad *Rub. id.*, 08. VII. 1869, KARSTEN 2696 (H).

The following description is based mainly on dried herbarium material. Some characters of living material are also included, because H. O. BARAL and B. DECLERCQ have kindly forwarded their drawings and notes for our study. Abbreviations of reagents: CB (cotton blue), CR (Congo red), MLZ (Melzer's reagent), IKI (Lugol's solution).

Apothecia mostly in swarms, more rarely densely crowded, not confluent, up to 1.2 mm in diam. when fresh, sessile on a narrowed central base or with a short and stout stipe which is often obscured by the wide discs, in one population more clearly stipitate; stipe up to 150(-250) µm long and 100(-200) µm wide.

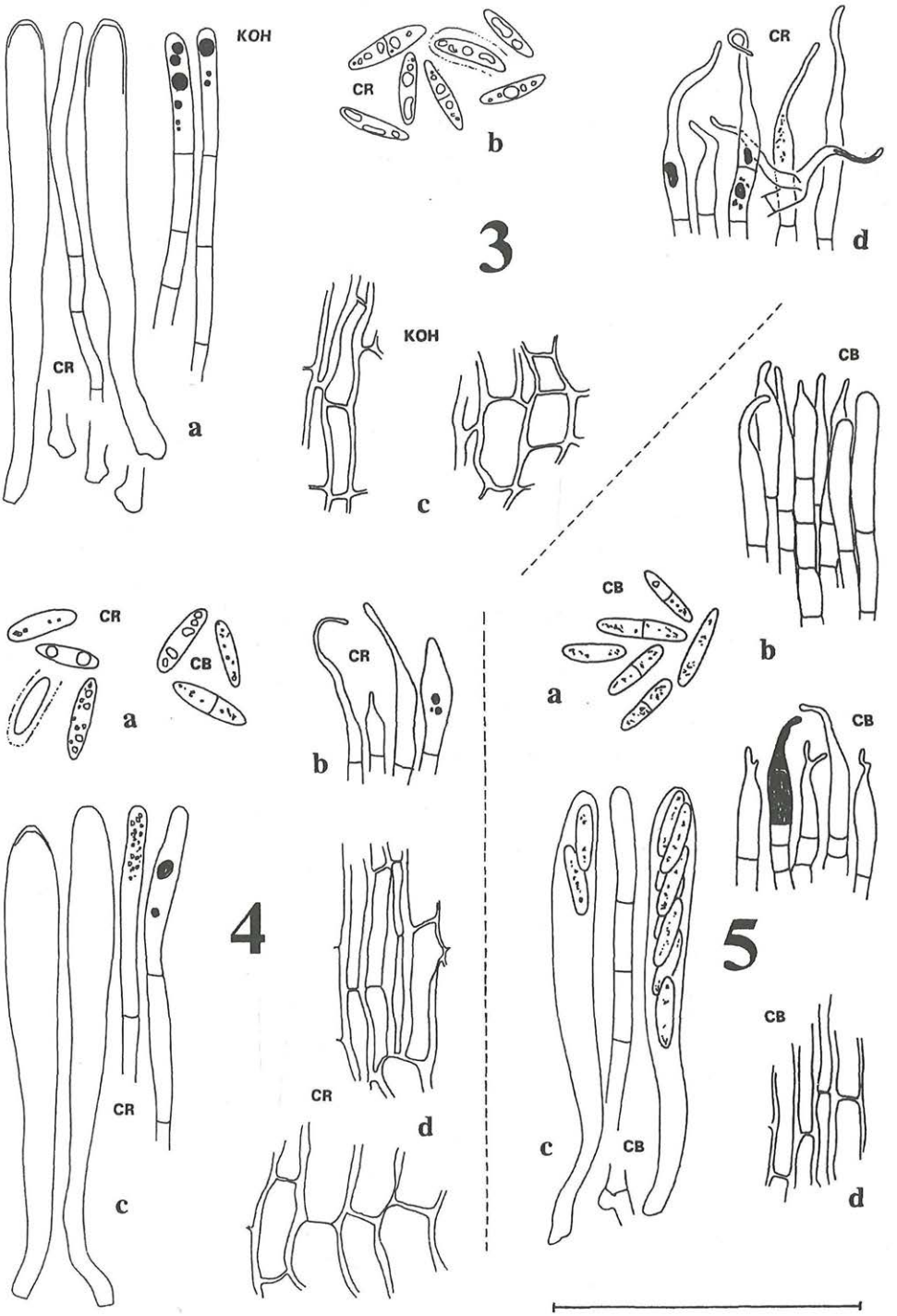
Disc orbicular to undulate-irregular in outline, mostly more or less flat or slightly convex with a shallow but distinct marginal rim, in a few populations more concave discs with inrolled margins have been noted; colour white, yellow to rarely orange-yellow when fresh.

Flanks concolorous with the disc or paler, pale cream when dried but often unevenly stained red-brown when dried, staining present also in fresh material when touched; hair cover varying between the populations from rather few, dispersed hairs to a dense cover of downy appearance.

Ectal excipulum of the flanks in longitudinal section consisting of somewhat undulating hyphae at a very low angle to the surface; degree of gelatinization varying between the populations from a textura prismatica (cells measuring 8-20 x 5-10 µm) with 1-2 µm thick glassy walls to a more rare type with equally gelatinized textura oblita; in one collection the hyphae were easily separated by squashing in CR; walls hyaline, not stained in CB, CR, MLZ; the cylindrical hyphal ends at the margin thin-walled, with rounded ends. In some populations the margin is mainly composed of such rounded hyphal ends and tapering hairs are very rare, in some populations tapering hairs form most of the margin.

Hairs with thin, smooth walls, straight to curved to hooked, in a few populations occasionally bifurcate, in one collection with typically cincinnate apices, mainly aseptate, more rarely one-septate, in squash mounts free hairs appearing one- to two-septate; apical cell ca. 12-32(-40) x 2.5-4.2(-5.0) µm, with a broad base, tapering continuously to often abruptly into a fine, 0.5-1.0 µm wide, non-solidified apex; resin lacking but hairs usually containing a yellow pigment; pigment appearing as faint yellow, globular vacuoles in KOH, as deformed bodies in other media, golden yellow in MLZ and CR, deep blue in CB.

Asci 68-105 x 7.3-10.5 µm when fresh in water (from 3 populations, number of observations unknown); 60-89 x 5.4-9.4 µm in CB, x=72.7 x 7.8 µm (n=30, from 10 populations), eight-spored, in one population rarely four- or six-spored, cylindric-clavate with a slightly conical apex, arising from croziers, pore MLZ+, IKI+ (blue); staining reaction seen as two thick lines through the whole apical wall.



Figs. 3-5. *Phialina separabilis*. - 3. BARAL 4185: (a) asci and paraphyses (b) ascospores (c) ectal excipulum in surface view (d) marginal hairs. - 4. DENNIS 19. IX. 1948: (a) ascospores (b) marginal hairs (c) asci and paraphyses (d) ectal excipulum in surface view. - 5. KRIEGER, Fungi Saxonici 1483: (a) ascospores (b) marginal hairs (c) asci and paraphyses (d) ectal excipulum in surface view. - Bar: 50 µm.

Ascospores 12.0-16.5 x 2.5-3.7(-4.0) µm when fresh in water (from 4 populations, number of observations unknown); 10.5-16.0(-18.0) x 2.5-4.0(-5.0) µm in CB, MLZ, x=13.0 x 3.2 µm (n=137, from 15 populations), ellipsoid-fusoid, smooth, hyaline, mostly with two subterminal groups of guttulae in CB, more prominently and evenly guttulate in CR, appearing aguttulate in MLZ, aseptate to one-septate, septum MLZ-; a gelatinous sheath (often?) present in fresh material, more rarely observed in herbarium material, then ca. 1.0-1.5 µm thick, staining faintly rosy in Cresyl blue.

Paraphyses cylindrical to slightly clavate, densely septate below the apical cell, dichotomous branching not typically seen above the level of ascal bases, terminal cells 18-44 x 2.7-5.0 µm in CB, x=30.2 x 3.6 µm (n=30, from 9 populations); pigment typically present in the apical cell, appearing as elongate or globose vacuoles when fresh in water, in dried material identical to the pigment inside the hairs.

Thus far the species has been collected solely from stems of *Rubus* species. Nearly all specimens are from *Rubus idaeus* except for BARAL 4255 (*R. caesius*), THAXTER (*Rubus* spec.) and ELLIS & EVERHART 1309 (*R. strigosus*). The species has been collected from July to November. Nearly 80 % of the collections have been made in August or September.

Material studied

Austria: Steiermark, Graz, Mariatrost, 29. IX. 1993, SCHEUER 2974 (GZU). - Stubalpe, SW of Köflach, valley of the Frei-Gössnitz-Bach, 800 m s. m., 26. IX. 1985, SCHEUER 1169 (Plantae Graecenses-Fungi 705; GZU, TUR). - Wölzer Tauern, 6 km SSW from Donnersbachwald, ca. 1300 m s. m., 16. VIII. 1986, SCHEUER 1632 (GZU, TUR). - Pölstal, W-slope of the Franz-Josephs-Höhe near Unterzeiring, 900 m s. m., 04. VIII. 1985, SCHEUER 1428 (GZU). - Kärnten, Nockgebiet, upper Gurktal W of Weitensfeld, Braunsberg S of Kleinglödnitz, 784-930 m s. m., 27. VIII. 1989, SCHEUER 2128 (GZU). - **Belgium:** La Roche, 27. VIII. 1989, DECLERCQ 89/092 (TUR). - **Canada:** Newfoundland, XI. 1898, WAGHORNE (Fungi Columbiani 1309; K). - **Finland:** Etelä-Häme, Tammela, Mustiala, IX. 1866, KARSTEN (lectotype of *H. separabile,* S). - ibid., 08. VII. 1869, KARSTEN 2696 (lectotype of *H. firmulum*, H). - **France:** Belfort - Montbéliard, E of La Cray, 20. IX. 1990, MARSON & BARAL 4255 (Herb. BARAL, TUR). - **Germany:** Bayern, Chiemgau, Eggstätt-Hemhofer Seenplatte between Hartmannsberg and Stefanskirchen, 26. VIII. 1987, SCHEUER 2098 (GZU). - Brandenburg, Niederbarnim, Klosterfelde, 22. IX. 1924, P. SYDOW (Mycotheca Germanica 2724; K). - Potsdam, NSG Fresdorfer Moor, 31. VII. 1968, BENKERT (BHU). - Berlin, Jungfernhaide, IX. 1885, P. SYDOW (Ascomyceten 811, two specimens; K). - Sachsen, Königstein, Bielagrund, 29. VIII. 1903, H. & P. SYDOW (Mycotheca Germanica 84; K). - Königstein, IX. 1888 and VII.-VIII. 1899, KRIEGER (Fungi Saxonici 1483; K). - **Luxembourg:** Syren, Alzingen, "Heid", 290 m s. m., 04. XI. 1989, MARSON (Herb. BARAL 3913, TUR). - **Sweden:** Gästrikland, Gävle, Lövudden, 12. VIII. 1956, NANNFELDT 14416 (Fungi exs. Suecici 3346; C, K). - **Switzerland:** Graubünden, Davos-Platz, Dischmatal, 1590 m s. m., 04. IX. 1990, BARAL, RAITVIIR & BLANK (Herb. BARAL 4185, TUR). - **UK:** England, Yorkshire, Pickering, Allerston, Givendale, 12. X. 1961, BRAMLEY K/63/4 (K). - Northern Ireland, Co. Down, Hillsborough, 17. IX. 1948 (K). - Scotland, Angus, Glamis (Herb. BERKELEY in K). - Dumfriesshire, Eskdale, Langholm, 19. IX. 1948, DENNIS (K). - Skye, Isle of Raasay, 7. IX. 1976, DENNIS (K). - **USA:** Maine, Kittery Point, IX. 1916, THAXTER (Reliquiae Farlowianae 124; K).

Discussion

Phialina separabilis is closely related to *P. ulmariae* (LASCH in RABENH.) DENNIS. The variability in the degree of excipular gelatinization is identical to that seen in *P.*

ulmariae (HUHTINEN 1990). Other characters which these species share include their occurrence on a non-woody substrate, the pigment, the structure of hairs, the elongated shape of the spores, ascal characters as well as the broadness and dense septation of the paraphyses.

The species has been placed close to the genus *Calycina* NEES ex GRAY by BARAL & KRIEGLSTEINER (1985) and WEBER (1992), apparently due to their overlooking the often sparse and inconspicuous hairs. WEBER (1992) suggested that this species would be closely related to *Calycellina* HÖHN., a genus which has also been treated as synonymous with *Phialina* by BARAL (1989).

One specimen in the cited material was determined as "cf. *separabilis*" only. BENKERT's collection showed a gelatinized, definitely hyphoid excipulum. In this respect this collection was at the extreme of the range of variation. In all other characters, however, it was identical to main material.

The type specimen of *Helotium firmulum* P. KARST. is apparently a somewhat juvenile collection of *P. separabilis*. The main characteristics are similar to this species, except that the asci and spores are somewhat smaller (Fig. 2). Their size deviates from the range of variation observed in other populations of *P. separabilis*, but this is most likely due to their juvenile condition.

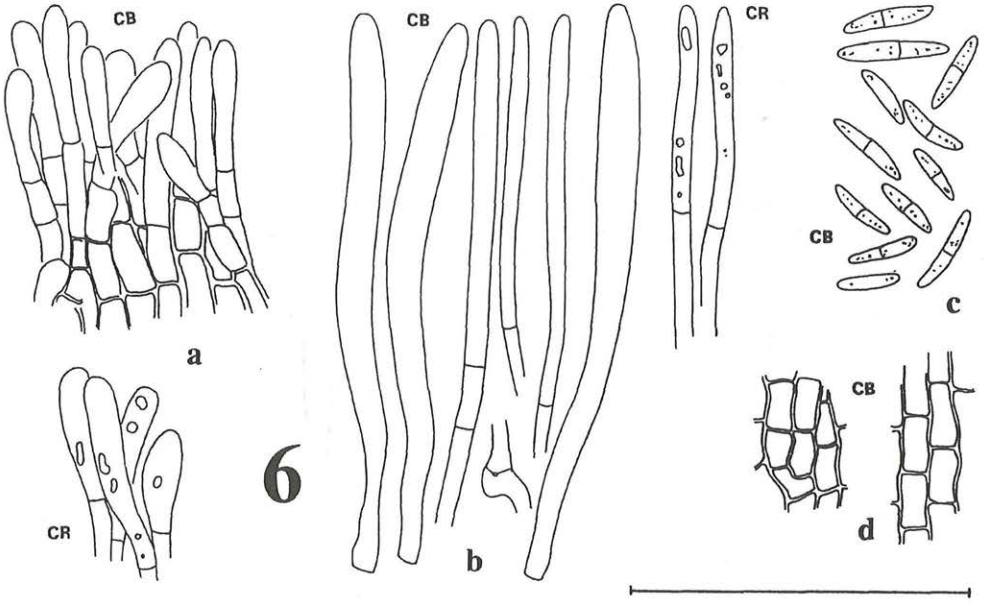


Fig. 6. *Calycina* spec. on *Teucrium scorodonia*, KRIEGER, Fungi Saxonici 1239: (a) marginal hyphal ends (b) asci and paraphyses (c) ascospores (d) ectal excipulum in surface view. - Bar: 50 µm.

DENNIS (1956) suggested that the material REHM (1896) cited as *Niptera dilutella* (FR.) REHM would be conspecific with the present fungus. REHM cited LINHART's (1883) Fungi Hungarici 158 as excellent material of the species and stated, on the basis of KARSTEN's diagnosis, that *Helotium separabile* was a distinct but closely related species. LINHART's exsiccatum seems to be somewhat heterogeneous. The set in BP was not on *Rubus* and those in HBG, M and WU showed no well-developed apothecia. Good material was seen in the set from TUR, but the taxon present was not closely related to *Phialina separabilis*.

KRIEGER (1897), REHM (1898) and SYDOW & SYDOW (1906) distributed a fungus on *Teucrium scorodonia* L. under the names *Pezizella separabilis* and *Belonium separabile*, resp., in their exsiccata (material studied given in parenthesis): KRIEGER, Fungi Saxonici 1239 (GZU, HBG, M), REHM, Ascomyceten 1217 (BP, BR, M, ZT), and SYDOW & SYDOW, Mycotheca Germanica 500 (BP, BR, C, E, HBG, MB, WU, ZT). They all apparently originate from the same locality. All sets show a fungus which quite closely resembles *Phialina separabilis* in having the same appearance, same pigments, quite similar spores, etc. This species can, however, be distinguished easily due to its ecology, longer and narrower asci, smaller spores, more regularly prismatic cells in the ectal excipulum and the margin composed solely of cylindric-clavate hyphal ends (Fig. 6). In cotton blue, the pigment inside the paraphyses and marginal cells seems to be totally dissolved, whereas in *P. separabilis* the pigment is often abundantly present. Only in CR the pigment could be observed in both taxa. HÖHNEL (1926), after studying KRIEGER's Fungi Saxonici 1239, concluded that the fungus was just a form of *Helotium herbarum* (PERS.: FR.) FR. This species on *Teucrium scorodonia* is best placed in *Calycina*. It is definitely not conspecific with *Niptera teucrii* FUCKEL (1872; Fungi rhenani 2378; G, holotype), nor with *Calloria leucostigmoides* SACCARDO (1880; PAD, holotype?), a taxon already synonymised with the former by REHM (1896).

We gratefully acknowledge the help of HANS OTTO BARAL (Tübingen) and BERNARD DECLERCQ (Wachtebeke, Belgium) who have sent us some recent collections and photocopies of their drawings of living material of *P. separabilis* and their notes to amend our description. Thanks are also due to the curators of all herbaria contacted for the loan of specimens, especially to Dr BRIAN M. SPOONER (R. B. G. Kew).

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