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Holmiella sabina (de Not.) comb. nov.
(syn. *Eutrybliidiella sabina*) and its *Cornicularia*-like
anamorph, an endophyte of *Juniperus* species

Orlando Petrini, Gary J. Samuels and Emil Müller

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Eutrybliidiella (Rehm) Hoehnel is a genus of the Patellariaceae Corda whose six species [*E. hysterina* (Dufour) Hoehnel, the type species, *E. araucariae* Butin, *E. panchananii* K.G. Mukerji & S. Dhwan, *E. sabina* (de Not.) Hoehnel, *E. torulispora* (Phillips) Dennis and *E. viburni* (Schweinitz) Groves] are united through having discoidal ascomata, bitunicate asci and brown, bicellular ascospores. Of the six species, only *E. hysterina* and *E. panchananii* are, possibly, congeneric, although we have not seen material of the latter species. *Eutrybliidiella araucariae*, because of its J+ ascus walls, can be referred to the Lecanorales near *Buellia* de Not. or *Karschia* Koerber. The affinities of *E. torulispora* and *E. viburni* are, at present, obscure.

Eutrybliidiella hysterina is very close to *Rhytidhysterium rufulum* (Spreng) Speg., the type species of *Rhytidhysterium* Speg., and could be placed in *Rhytidhysterium* which is the older genus. The ascomata of both species arise from a thin, effused, subcortical stroma. They are hysteriform, usually linear to triangular, transversely costate and they open to become discoidal by regular unfolding of the lips of the ascomatal slit. On drying, the lips refold and the ascoma once again assumes a hysteriform aspect. An epithelial layer is formed of the disintegrating tips of paraphyses embedded in an amorphous substance that does not react to 3% KOH. The paraphyses are infrequently branched and are enclosed in a J+, gelatinous sheath; the iodine reaction is reversible in 3% KOH. It is difficult to separate asci from the plexus formed by the paraphyses. Asci are bitunicate, J– and cylindrical to narrowly clavate. Ascospores are opaque, dark brown to black and lack terminal pores; they are uni-septate in *E. hysterina* and triseptate in *R. rufulum*. Tissue of the medullary excipulum is pseudoparenchymatous. Anamorphs of *E. hysterina* (Baumeister 1957, as *Trybliidiella hysterina* (Dufour) Shear; Urries 1950, as *Trybliidiella elevata* (Pers. ex Fr.) Rehm, personal observations) and *R. rufulum* (Shear 1933, Voorhees 1939 as *Trybliidiella rufula* (Spreng) Sacc., personal observations) are *Phoma* and *Diplodia*.

Eutrybliidiella hysterina is found only on *Buxus sempervirens* L. and *B. papillosa* C.K. Schneider and is found in southern Europe and India. *Rhytidhysterium* is a genus of two or three, saprobic or weakly parasitic species which are found on a wide variety of woody plants at tropical and subtropical latitudes. *Eutrybliidiella sabina* is easily distinguished from *E. hysterina* and *R. rufulum*. Ascomata of *E. sabina* are circular to elliptic, rarely elongated and are at first completely enclosed. They open by irregular splitting of the surface layer into three to four toothlike lobes that fold back to reveal a disc but do not reclose upon drying. The ascomata form entirely within cortical tissue and do not arise from a stroma. An epithelial layer is formed by the tips of paraphyses which are embedded in a black, amorphous substance. This substance is brown in 100% lactic acid and green in 3% KOH, a reaction that is reversible. The J- paraphyses are red in 3% KOH and subhyaline in 100% lactic acid but this reaction is not reversible and KOH does not affect paraphyses which were first immersed in 100% lactic acid; they are not enclosed within a gelatinous sheath. The bitunicate asci are J- and broadly clavate. Ascospores are opaque, dark brown to black and equally bicellular with a thin, poroid area at each end of each spore. Tissue of the medullary excipulum is hyphal. The anamorph of *E. sabina* is *Corniculariella*-like with phialogenous, stylosporous, hyaline, aseptate conidia. *E. sabina* is found only on *Juniperus* spp. and is widely distributed in Europe, Asia and North America (Holm & Holm 1977). *E. sabina* is clearly not congeneric with *E. hysterina*, differing in features of ascomatal morphology, anatomy, reactions to Melzer's reagent and KOH, ascospores and anamorph.

E. sabina has been placed in a wide variety of genera, none of which are satisfactory for the species. Rehm (1896) drastically emended *Caldesia* Trevisan (Trevisan 1871) by specifically excluding all six of the original species and retaining the genus for the sole species *C. sabina* (de Not.) Rehm. He therefore effectively described the new genus *Caldesia* Rehm based on *C. sabina* which is illegitimate because it is a later homonym of *Caldesia* Trevisan.

No type species has been designated for *Caldesia* Trevisan and Rehm (1896) stated that the original species belong in part to the „Arthonieen“ and in part to *Melaspilea* Nyl. Whatever species is eventually chosen to typify *Caldesia* Trevisan is irrelevant to *Eutrybliidiella sabina* since, based on descriptions, it is not congeneric with any of these species. We have been able to follow the fate of only three of the original species. *Caldesia melaleuca* (Fr.) Trevisan is treated in *Arthonia* Ach. and *C. rugulosa* (Krempelhuber) Trevisan in *Allarthonia* (Nyl.) Zahlbruckner by Redinger (1937). *Caldesia inconspicua* (Babington) Trevisan (= *Myriangium inconspicuum* Babington) was placed in synonymy with *Arthonia lurida* Ach. by Nylander (1858) and an isotype of *C. ephelodes* (Nyl.) Trevisan (= *Arthonia ephelodes* Nyl.) in ZT has no fruiting structures. This species has not been redisposed and neither have *C. didyma* Trevisan and *C. proximella* (Nyl.) Trevisan (= *Lecidia proximella* Nyl.). We have been unable to evaluate *C. salutaris* Teng which Pirozynski and Reid (1966) suggested might be a species of *Eutrybliidiella* in the sense of *E. sabina*, certain features of which have incorrectly come to characterize the genus *Eutrybliidiella* (cf. von Arx & Müller 1975).

Eutrybliidiella sabina cannot be accommodated in any of the genera of the Patellariaceae Corda (*sensu* von Arx & Müller 1975), or any of the lichenized, ascomycetous genera. We accept Rehm's (1896) argument that *Tryblidium sabinum* de Not. should

be placed in its own genus and, since *Caldesia* Rehm is illegitimate, we propose the new generic name *Holmiella* for *T. sabinum*.

The affinities of *Holmiella* are obscure. It does not seem to be closely related to *Eutrybliidiella hysterina* which is close to dothideaceous genera such as *Botryosphaeria* Ces. et de Not. There are no obvious close relatives within the Patellariaceae, a family whose members are brought together only because of their apothecioid ascomata and bitunicate asci. Through its *Corniculariella*-like anamorph the discomycetous genera *Dermea* Fr. and *Durandiella* Seaver are suggested (DiCosmo 1978), but the asci of these species are unitunicate and in *Dermea* the ascus apex is J+. The reaction of the epithecium to KOH and the stylosporous conidia indicate a relationship to lichenized genera such as *Arthonia*.

It is difficult to satisfactorily place the anamorph of *H. sabina* into any of the known genera of fungi imperfecti. The closest morphological comparison that we can make is with *Corniculariella* Karst. em. DiCosmo or with *Foveostroma* DiCosmo, also anamorphic to dermatiaceous discomycetes. This anamorph is like these two genera in having non-stromatic, cupulate pycnidia and long, slender conidia produced from phialides that may arise from branching conidiophores. It differs in having unicellular conidia and from *Foveostroma* in having unilocular conidiomata although pycnidia produced in culture may have a labyrinthiform locule such as is found in *Foveostroma*. Even though the relationship to *Corniculariella* and *Foveostroma* may not be biologically based, we feel that the form of the conidiomata of *H. sabina* is well accounted for in *Corniculariella*.

During a thorough investigation on endophytes of *Juniperus communis* L. (Petrini 1978) we have regularly isolated *H. sabina* from living needles and woody twigs, collected at different localities in Switzerland and France (Table 1). These isolates from the living apparently healthy tissue of *J. communis* were identical to isolates derived from solitary ascospores in cultural characters, conidiomata and conidia. Since the tissue from which isolates were made was surface-sterilized, since neither conidiomata nor ascomata were seen and since there was no outward sign of infection of these plant parts, we conclude that *H. sabina* is an endophytic fungus within living tissues of *Juniperus communis* and probably the other species of *Juniperus* from which ascomata of *H. sabina* have been reported. We cannot easily describe the nature of the relationship between *H. sabina* and *J. communis*.

Mycelium is isolated from within symptomless tissue while fructifications are found some distance from the apparently healthy tissue on dead branches. *Holmiella sabina* has never been reported from needles of *Juniperus* spp. nor has a conidial fungus resembling its anamorph been described from *Juniperus*. It seems likely that the fungus is always present within living tissue either as a parasite, in that it derives its nourishment from the *Juniperus* while giving nothing in exchange, or in some undetermined way as a commensal. This connection with *Juniperus* explains its host specificity, which is not easily understandable when only the teleomorph growing on apparently dead tissue is considered. However it seems that ascomata are formed soon after the death of the twigs which even may contain some still living portions of tissue. We cannot say whether *H. sabina* merely sporulates in response to previously killed tissue. The observations described here help to explain the seeming host specificity of many „saprophytic“ fungi.

A redescription of *Holmiella sabina* follows.

Table 1:

Number of records of *Corniculariella*-anamorphs of *Holmiella sabina* in 5 different collecting localities in Switzerland (Montaccio di Cademario TI, Landquart GR, Weiach ZH, Hesseberg AG) and two collecting localities in France (Musièges, Savoie and Uzès, Provence). In brackets: Number of investigated needles.

SWITZERLAND		FRANCE	
Needles	Twigs	Needles	Twigs
51 (4229)	13	— (108)	1

Materials and Methods

Single ascospores were isolated onto 2% malt extract agar (ME) with the aid of a micro-manipulator. Isolated ascospores were allowed to germinate for ca. 12 hrs at 20–23°C. Cultures were studied on oatmeal agar (OA, Difco), cornmeal agar (CM, Difco), Cornmeal + 1% dextrose agar (CMD) and potato dextrose agar (Difco) + 0.5% yeast extract (PDYE, Difco yeast extract). Colonies were incubated for two weeks at 18–23°C with alternating 12 hrs darkness and 12 hrs „Grolux“ (Sylvania) light.

To study endophytism, needles of various age classes were collected and isolations were made within 48 hrs. Needles and twigs were surface sterilized following the method of Carroll et al. (1977) with some modification of sterilization times. Needles and twigs were dipped for 1 min in 96% ethanol to wet the surface. They were then immersed for 5 min in a solution consisting of 2 parts 14% v/v (aq) sodium hypochlorite: 1 part water, then reimmersed in 96% ethanol for 30 sec. Needles were then cut into 2 segments and the twigs into pieces 3–5 mm long and transferred to Petri dishes containing ME. The plates were incubated at 18°C and periodically checked for fungal growth, which was then isolated.

Holmiella Petrini, Samuels et E. Müller nom. nov.

= *Caldesia* Rehm, Rabh. Kryptogamenflora 1 (3): 290. 1896.
non Trevisan, Hedwigia 10: 151. 1871.

Ascomata apotheciis habitu similia, primum clausa demum autem irregulariter secedentia superficie ascomatis, stromate non praedita. Asci bitunicati, iodo non reagentes. Ascospores atrobrunneae vel nigrae. Paraphyses filamentosae, ramicantes superque apices ascorum dense reticulatim anastomosantes; paraphysum apices gelatinam copiosam 3% KOH viridescentem percurrentes.

Species typica: *Holmiella sabina* (de Not.) Petrini, Samuels et E. Müller.

Ascomata apothecioid, at first closed but opening by irregular splitting of the upper surface of the ascomatal wall, non-stromatic. Asci bitunicate, J-. Ascospores dark brown to black. Paraphyses filamentous, branching and anastomosing above ascus tips to form a dense reticulum; tips of paraphyses embedded in an amorphous substance that becomes green in 3% KOH.

Etymology of the generic epithet. Refers to Drs. Kerstin and Lennart Holm, the University of Uppsala, in recognition of their work on the fungi inhabiting *Juniperus* spp.

- Holmiella sabina* (de Not.) Petrini, Samuels et E. Müller, comb. nov. (Figs. 1–3).
- = *Tryblidium sabinum* de Notaris, Comment. Soc. Critt. Ital. 2: 491. 1867.
 - = *Karschia sabina* (de Not.) Rehm, Hedwigia 21: 115. 1882.
 - = *Caldesia sabina* (de Not.) Rehm, Rabenh. Kryptogamenflora 1 (3): 290. 1896.
 - = *Eutrybliidiella sabina* (de Not.) Hoehnel, Sitzb. Akad. Wiss. Wien, Math.-Naturwiss. Kl. 127: 564. 1918.
 - = *Trybliidiella sabina* (de Not.) Nannfeldt, Nova Acta Soc. Sci. Uppsala, Ser. 4, 8 (2): 334. 1932.
 - = *Cenangium deformatum* Peck, Bull. N.Y. State Museum 28: 68. 1876.
 - = *Cenangella deformata* (Peck) Saccardo, Syll. Fung. 8: 593. 1889.
 - = *Phaeangella deformata* (Peck) Saccardo et Saccardo, Syll. fung. 18: 128. 1906.
 - = *Karschia deformata* (Peck) Peck, Bull. N.Y. State Museum 137: 117. 1909.
 - = *Dermatella deformata* (Peck) Seaver, The North American Cup Fungi (inoperculates). New York, p. 313. 1951.
 - = *Diplodia kansensis* Ellis et Everhart, Proc. Acad. Nat. Sci., Philadelphia 1894: 363.
 - = *Trybliidiopsis occidentalis* Earle, in Greene's Plantae Bakerianae, Washington 2 (1): 9. 1901.

Anamorph: (= imperfect state) *Corniculariella* Karsten emend. DiCosmo.

Teleomorph: (= perfect state)

Ascomata apothecial, circular to elongate, rarely triangular, 0.5–1 mm diam., solitary to gregarious in groups of a few, at first pulvinate and covered with a continuous outer layer, outer layer splitting irregularly into 3–4 ± triangular lobes that fold back to expose a black hymenium; produced entirely within tissue of bark, breaking through surface of bark; no stromal formation. Hymenium black when fresh and when dry. Receptacle black, smooth, slightly shining. Ascomata sessile. In 3% KOH no soluble pigment or sometimes a red pigment seen.

Asci bitunicate, 4–8-spored, (85–)105–125(–135) x 30–45 µm, clavate to broadly clavate, base abruptly truncated; apex broad, wall thickened, with a broad „nasse apicale“; ascus wall and apical apparatus J–.

Ascospores dark brown to black, opaque, (25–)29–37(41) x (11–)13–18(–22) µm, bicellular, with a pore in the septum, equally 2-celled or one cell slightly longer, a single drop in each cell, elliptic, not constricted at the septum, each end of each ascospore poroid (see notes below), surface of spore minutely pitted. Germinating within 5 hrs, producing a single, unbranched, 15–25 µm long germ tube from each end of each ascospore. Paraphyses 25–50 µm longer than asci, branching and anastomosing frequently above to form a reticulum, branching less frequently below, septate,

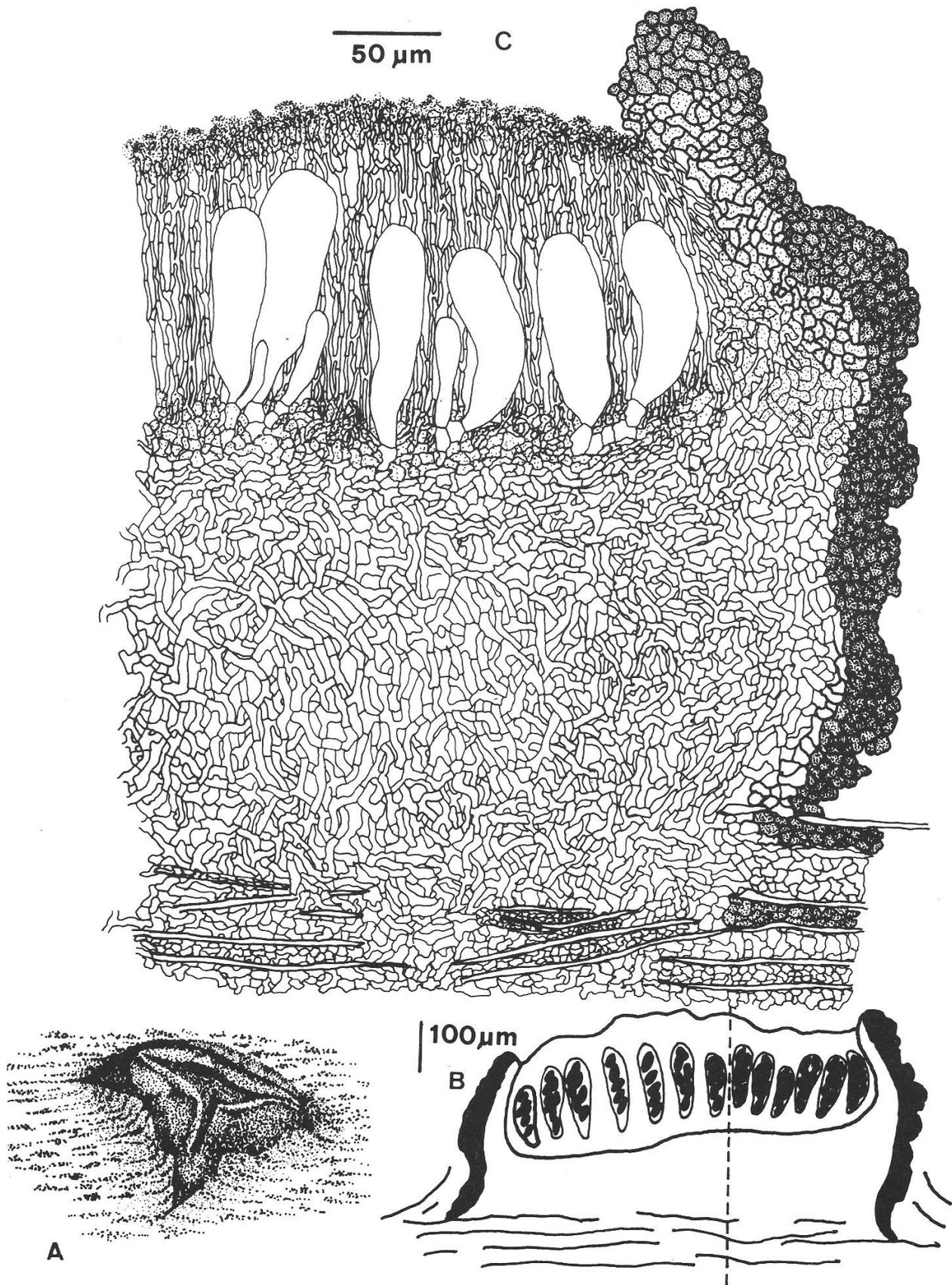


Fig. 1:

Holmiella sabina. A. Habit sketch of ascoma. B. Diagram of median, longitudinal section of ascoma. C. Detail of portion of fig. 1 B right of the dashed line (Fungi of West Pakistan 15622).

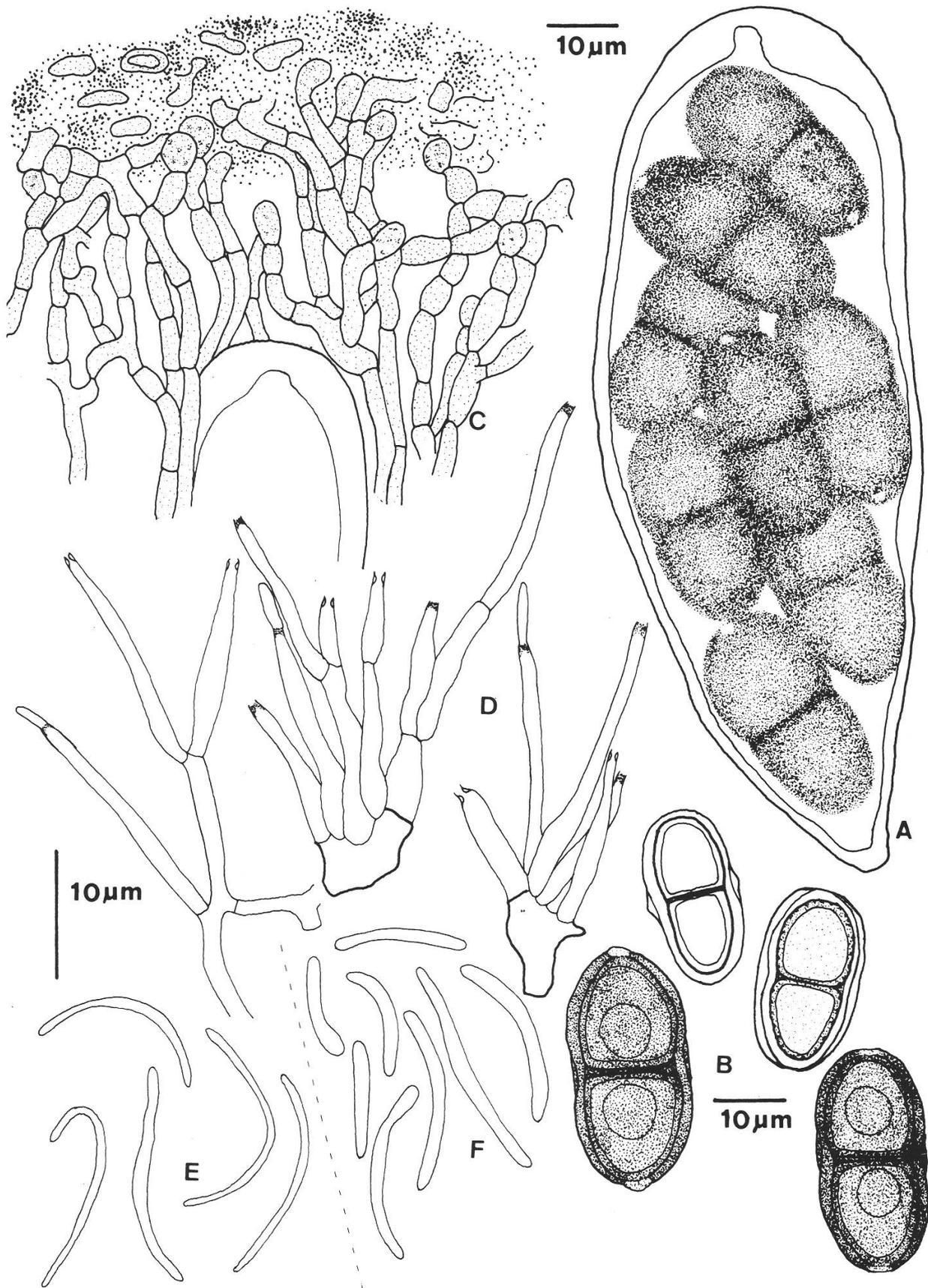


Fig. 2:

Holmiella sabina. A. Ascus. B. Stages in development of ascospores (lactic acid, bright-field microscopy). C. Portion of Epithecium. D. Phialides produced in culture (Lactic acid, phase-contrast microscopy). E. Conidia produced in culture. F. Conidia produced in nature. (A–C: Fungi of West Pakistan 15622; D, E: GJS 78–54; F: Bramant).

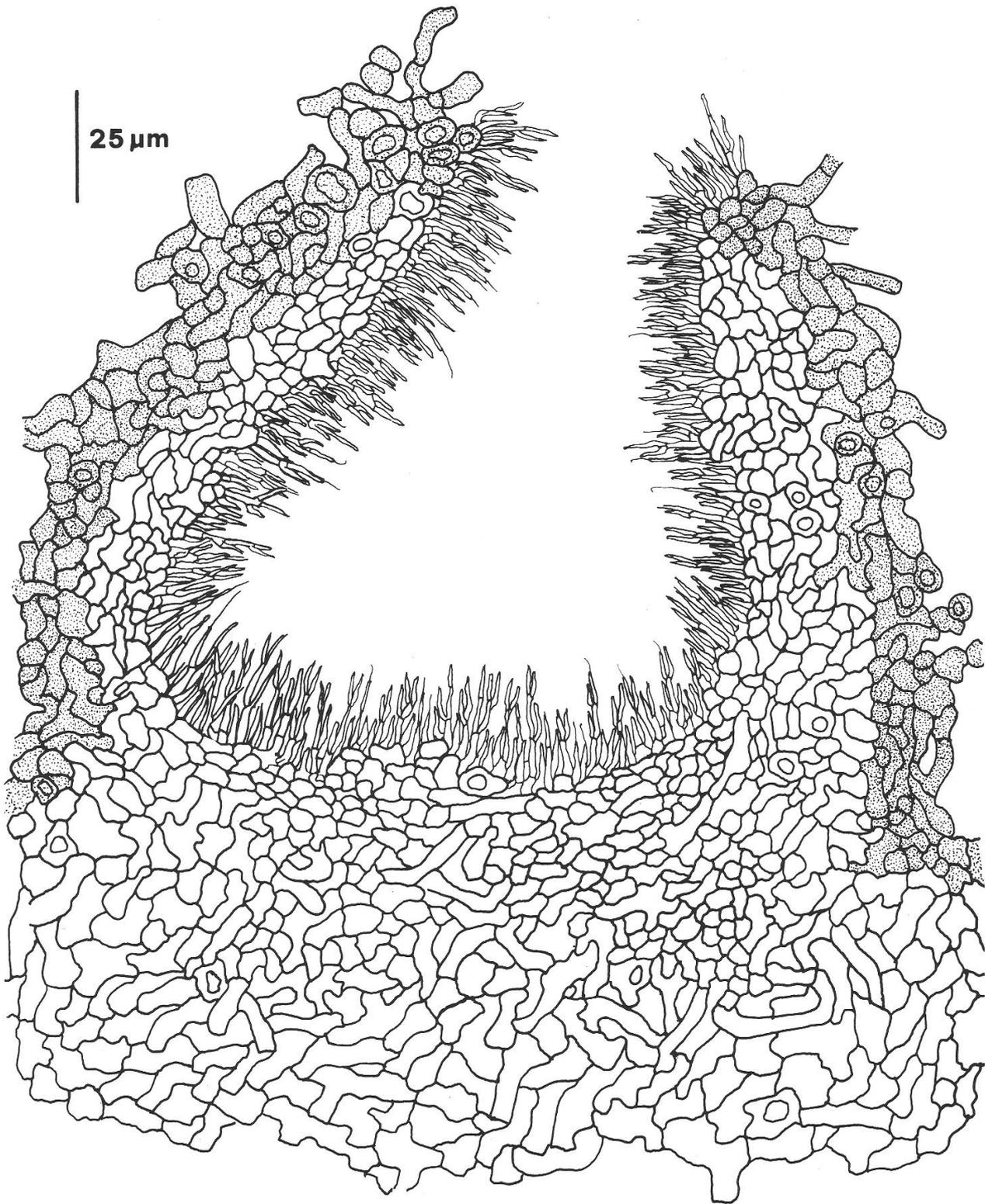


Fig. 3:
Holmiella sabina: Median longitudinal section of pycnidium produced on OA (GJS 78-54).

3–3.5 μm wide, lightly brown pigmented, slightly spinulose above, filiform, short-celled above with tip cells subglobose, 4–5 μm diam.; becoming red in 3% KOH and pale brown in 100% lactic acid, reaction not reversible. Tips of paraphyses embedded in a continuous layer of black, anamorphous substance which turns green in 3% KOH and brown in 100% lactic acid, reaction reversible.

Subhymenium formed of bases of paraphyses and larger, pseudoparenchymatous ascogenous cells; immediately below ascus bases a thin, dense region of \pm horizontally oriented, branching, hyphal cells; subhymenium lightly brown pigmented. Medullary excipulum comprised of loosely intertwined, branching, \pm vertically oriented, septate, 3–4 μm wide hyphae; walls slightly pigmented, smooth, subhyaline, arising directly from the cells growing in the cortex. Toward the outer edge of the ascomata cells becoming compacted, *textura epidermoidea*. Margin of the disc comprised of prosenchymatous, light brown cells outermost 10–15 μm of the ascomatal wall very heavily pigmented and walls thickened.

Characteristics in culture.

Colony characters: Colonies on OA, CM, CMD, PDYE 1–2.5 (OA) cm diam, no aerial mycelium, submerged mycelium black.

Conidiomata pycnidial, globose, ca 200 μm diam, black, superficial or immersed; on OA, CM and CMD forming in caespitose groups of from two to several, on PDYE the entire surface of the colony raised and forming pycnidia in a continuous crust. Pycnidia opening by splitting of the upper surface, wall ca 50 μm wide, composed of *textura epidermoidea*, cells of outer ca 25 μm heavily pigmented.

Phialides cylindrical to sub-cylindrical, 10–17 μm long, tapering from 1.5–2 μm wide basally to 1–1.5 μm wide at the unflared opening, solitary or in pairs, arising directly from cells of the wall or terminally and laterally from short conidiophores; conidiogenous cells arising from the entire inner wall of the conidioma.

Conidia stylosporous, 15–19 x 0.5–1 μm , straight to sharply curved, unicellular, hyaline, extruded from pycnidia in hyaline slime.

Habitat.

Ascomata and conidiomata found on branches of *Juniperus bermudiana* L., *J. communis* L., *J. macropoda* Boiss., *J. oxycedrus* L., *J. sabina* L., *J. scopulorum* Sarg., *J. virginiana* L., and from tissues of needles and wood of *J. communis*.

Illustrations. Müller & von Arx (1962, fig. 91 as *Eutryblidiella*), Holm & Holm (1977, fig. 5a, as *Eutryblidiella*) Pirozynski & Reid (1966, figs. 1–10, as *Eutryblidiella*), Rehm (1896, p. 283, figs. 1–5, as *Caldesia*).

Specimens examined. WEST PAKISTAN: Ziarat, on branches of *Juniperus* sp., S. Ahmad, 26.2.1962 (*Fungi of West Pakistan 15622*, ZT). FRANCE: Hautes Alpes, Aguilles (Val Queyras), on *Juniperus sabina*, Scheinpflug, 25.5.1958 (ZT); Hautes Alpes, Forsthaus Les Sauvas ob Mont-maur, on *Juniperus communis*, H. Kern, 24.4.1952 (ZT); Hautes Alpes, oberes Durancetal, Argentières, on *Juniperus sabina*, E. Müller, 23.6.1958 (ZT); Savoie, Bramans, Maurienne, on *Juniperus communis*, E. Müller, 28.6.1966 (ZT); Savoie, oberhalb Lanslevillard, on *Juniperus communis*, Egger, 1.7.1966 (ZT); Var, Massif de la Ste. Baume, Hôtel Miremonts, on *Juniperus oxycedrus*, E. Müller, 7.6.1966 (ZT); Vaucluse, Forêt de St. Lambert, on *Juniperus oxycedrus*, E. Müller, 25.5.1962 (ZT). SWEDEN: Dolby Parish, Jerusalem, Uppland, Uppsala-Näs, on *Juniperus communis*, O. Petrini & K. Holm, 28.8.1978 (GJS 78-54: PDD, ZT). USA: Kansas, Rooks Co., Rockport, on *Juniperus virginiana*, Bartholomew 1292, 7.12.1893 (Holotype *Diplodia kansensis*, FH).

Notes: The ascospore wall of *H. sabina* is composed of at least four layers with an epispore and endospore, each of two layers. The two layers of the epispore are usually evident at the septum. Pigmentation is first evident in the inner layers of the wall while the epispore is still hyaline. As the spore matures, the epispore also becomes brown, The ends of the spore remain hyaline and thinwalled, often the ends are slightly outwardly bulged to form a colorless cap. The outermost layer of the endospore, immediately below the lightly colored epispore, appears very thin and partially disintegrated, this development occurs as the spore matures since the wall of young spores is entire. Germ-tubes emerge only through the ends of the spore and the spore wall surrounding the emergent tube has a ragged, torn appearance suggesting that the ends of the spore are thin-walled rather than being pierced by a pore that is open to the exterior. It is often very difficult to see the poroid regions of the spores and this can be explained if these regions develop concomitantly with spore germination.

Pycnidia were found on one of the specimens cited above (FRANCE: Savoie, Bramans). They appeared as small, barely erumpent, hemispherical, black spots that split irregularly to expose a disc.

Pycnidia in longitudinal section are cupulate, ca. 260 μm wide x ca. 200 μm high. The pycnidial wall is ca. 30 μm wide, pigmented throughout and composed of cells nearly circular in outline, 7-10 μm in diameter with walls ca. 2 μm wide. Phialides were identical to these produced in culture but the conidia were shorter and broader, 11-14 x 0.5-1 μm .

Summary

The type species of *Eutrybliidiella* (Rehm) Hoehnel, *E. hysterina* (Dufour) Hoehnel is found to be a species of *Rhytidhysterium* Speg. *Eutrybliidiella hysterina* and *E. sabina* are not congeneric and *Holmiella* nom. nov. (= *Caldesia* Rehm. non Trevisan) is proposed with *H. sabina* (de Not.) comb. nov. (= *Tryblidium sabinum* de Not.) the type species. The connection between *Holmiella sabina* and a *Corniculariella*-like anamorph is proven through isolation of single ascospore. *Holmiella sabina* is an endophyte living within needles and wood of *Juniperus communis* L. and fruiting on dead tissues of *J. communis* and other *Juniperus* species.

Zusammenfassung

Die Typusart von *Eutrybliidiella* (Rehm) Hoehnel, *E. hysterina* (Dufour) Hoehnel, gehört nach neuesten Untersuchungen in die Gattung *Rhytidhysterium* Speg. *Eutrybliidiella hysterina* und *E. sabina* gehören jedoch nicht in dieselbe Gattung, weshalb der Gattungsname *Holmiella* als Ersatz für *Caldesia* Rehm non Trevisan vorgeschlagen wird; *H. sabina* (de Not.) comb. nov. ist Typusart. Die Zusammengehörigkeit von *Holmiella sabina* und einem *Corniculariella*-ähnlichen Anamorph konnte dank Kulturen aus einzeln isolierten Ascosporen erbracht werden. *Holmiella sabina* ist ein endophytischer Pilz im Innern von lebenden Nadeln und Zweigen von *Juniperus communis* L. Die Fruktifikation erfolgt auf totem Gewebe von *J. communis* und anderen *Juniperus*-Arten.

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