Life-History Studies of Brazilian Ascomycetes. 7.[1])

Rhytidhysteron rufulum and the genus *Eutryblidiella*

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Zusammenfassung. Die Umschreibung von *Rhytidhysteron rufulum* (Sprengel ex Fr.) Speg. konnte dank Kulturversuchen durch den Einbezug der *Diplodia*- und *Aposphaeria*-artigen Anamorphe erweitert werden. Nach den Typus-Kollektionen der zwei ursprünglichen Arten der Gattung, *Rh. brasiliense* Speg. und *Rh. viride* Speg. sind diese mit *Rh. rufulum* synonym. Weitere sechs, später beschriebene Arten von *Rhytidhysteron* sind auf Grund der an zahlreichen Kollektionen aus dem ganzen Verbreitungsgebiet der Pilze gewonnenen Erkenntnissen über die Variabilität der Merkmale nach ihren Beschreibungen ebenfalls synonym zu *Rh. rufulum*. Innerhalb des Lebenszyklus der sich nur in der Ascosporen-Septierung unterscheidenden *Eutryblidiella hysterina* (Dufour) Petr. [Typus von *Eutryblidiella* (Rehm) Höhnel] treten ganz ähnliche *Diplodia*- und *Aposphaeria*-artige Anamorphe auf; der Schluss, *Eutryblidiella* und *Rhytidhysteron* zu vereinigen und die Umschreibung von *Rhytidhysteron* so zu erweitern, dass auch didymospore Vertreter eingeschlossen sind, liegt nahe und wird vorgeschlagen.

Introduction

Rhytidhysteron Spegazzini is a genus of the Patellariaceae (sensu Arx & Müller 1975), a poorly known family of Ascomycetes whose members have discoidal ascomata and bitunicate asci. Ascomata of *Rhytidhysteron* are distinctive, being at first hysteriform, but opening along a preformed longitudinal cleft to become discoidal at maturity. Under dry conditions, the margin of the disc folds over and the ascomata once again become hysteriform, triangular or triradiate. They are also distinctive in that the paraphyses are enclosed in a gelatinous sheath which is usually iodine positive.

Although the generic name was originally published as *Rhytidhysteron* (Spegazzini 1881), Spegazzini later (1888) adopted without comment the spelling "*Rhytidhysterium*", the name that is used today. This orthographic change is not justified (International Code of Botanical Nomenclature 1972, Art. 73) and the original spelling *Rhytidhysteron* must be retained. Two species, *R. brasiliense* Spegazzini and *R. viride* Spegazzini, were originally included in the genus. Spegazzini did not designate a type; the first to do so were Clements

[1]) Part 6 in Sydowia 31: 180—181. 1978 (1979).

& SHEAR (1931), who selected *R. brasiliense* which PETRAK (1962) synonymized with *R. rufulum* (SPRENGEL ex FRIES) SPEGAZZINI (≡ *Hysterium rufulum* SPRENGEL ex FRIES).

A total of nine species have been included in the genus: *R. beccarianum* (CESATI) BATISTA & VALLE in BATISTA & MAIA, *R. brasiliense*, *R. discolor* (SPEGAZZINI) SPEGAZZINI, *R. guaraniticum* SPEGAZZINI, *R. javanicum* (PENZIG & SACCARDO) E. MUSSAT, *R. prosopidis* PECK, *R. rufulum*, *R. scortechnii* SACCARDO & BERLESE and *R. viride*. We studied the type specimens of *R. brasiliense* and *R. viride* and found them to be *R. rufulum*. Based on published descriptions, the remaining species are also *R. rufulum*.

Rhytidhysteron rufulum is common in the tropics and subtropics where it grows as a saprobe or weak parasite on wood of a wide variety of dicotyledonous plants. It is an attractive species, typically having

Table 1. Frequency distribution of means of ascospore lengths from 102 collections of *Rhytidhysteron rufulum*

Ascospore length (µm)	18—	20—	22—	24—	26—	28—	30—	32—	34—	36—	38—	40—
Black pseudoepithecium (n = 28)	0	0	1	0	1	0	7	8	5	5	0	1
Red pseudoepithecium (n = 77)	1	3	11	16	11	7	14	11	3	0	0	0

a bright, brick-red pseudoepithecium (KORF 1973). However, the surface of the pseudoepithecium may be deep purple or black. In most ascomata where the pseudoepithecium is black on the surface, it is bright red internally in a region immediately above the ascal tips; in fewer ascomata it is black throughout. This variation in color suggests that two species or varieties could be involved.

Mean dimensions of ascospores from one typical ascoma were determined for each of 105 collections from around the world; they ranged from 20×7.5 µm to 40×14.5 µm, all with similar length/breadth ratios. Of the 28 ascomata having completely black pseudoepithecia, all except 2 gave a mean ascospore length >30 µm. The 77 ascomata with completely or partially red pseudoepithecia gave a bimodal distribution of mean ascospore lengths (Table 1); the range of the upper mode was similar to the range of the mean ascospore lengths from forms with a black pseudoepithecium. Among the specimens examined, some had intermingled black and "small-spored" red pseudoepithecial forms, and others had intermingled black and

"large-spored" red pseudoepithecial forms. Moreover, there were no differences in the morphology and anatomy of the variously colored ascomata. The color of the pseudoepithecium is apparently variable within *R. rufulum* and is not closely correlated with ascospore size. We do not accept that two taxa can be distinguished on this basis.

VORHEES (1939) was the first to fully describe the life-cycle of *R. rufulum* (as *Tryblidiella*). He accepted two species, *T. rufula* (*Sprengel* ex FRIES) SACCARDO and *T. fusca* (ELLIS & EVERHART) REHM, on the basis of color of the pseudoepithecium. Each produced non-stromatic, *Diplodia*-like and *Aposphaeria*-like pycnidia. He described minor cultural differences between red and black forms but was not able to differentiate between the pycnidia of the respective anamorphs. We studied VORHEES' collections (NY) and found all to have superficially or internally red pseudoepithecia; hence he did not study a truly black form. As far as we are aware, a black form has not yet been grown in pure culture and we have not had the opportunity to do so.

SHEAR (1933) reported finding both *Diplodia*-like and *Aposphaeria*-like pycnidia in cultures derived from single ascospores of *R. rufulum* (as *Tryblidiella*). However, it is likely that he either did not actually isolate spores of *R. rufulum*, or had a mixed culture, since the *Diplodia*-like stage that formed in his cultures had brown, striate conidia suggestive of *Botryodiplodia theobromae* PATOUILLARD.

The taxonomic relationships of *Rhytidhysteron* are obscure. The genus does not have any close relatives in the heterogeneous Patellariaceae. The presence of an iodine positive sheath around the paraphyses suggests a relationship to the Lecideaceae (DENNIS 1977, HAFELLNER 1979), a family of lichenized fungi. Many species of the Lecideaceae are said to have a "hymenium" blued by iodine. *Rhytidhysteron* has biological affinities to both the Pleosporaceae (sensu ARX & MÜLLER 1975), through *Otthia* NITSCHKE, and the Botryosphaeriaceae. Like *R. rufulum*, the species of *Otthia* and the Botryosphaeriaceae are saprobes or weak parasites on wood of dicotyledonous plants, and they have bitunicate asci and *Diplodia*-like anamorphs. It is none the less difficult to reconcile the divergent ascomatal morphologies of these fungi may indicate a common response to similar conditions of substrate and environment.

Eutryblidiella (REHM) HÖHNEL (HÖHNEL 1918, ≡ *Tryblidiella* SACCARDO subg. *Eutryblidiella* REHM, 1904), with *E. hysterina* (DUFOUR) PETRAK as its type species, is generally considered to be the didymosporous counterpart of *Rhytidhysteron* (HÖHNEL 1918, PETRAK 1959, MÜLLER & ARX 1962, PIROZYNSKI & REID 1966). NANNFELDT (1932) took up *Tryblidiella* and listed *Rhytidhysteron* and *Eutryblidiella* as synonyms, thus accepting two spore morphologies in the genus. MÜLLER & ARX (1962) and ARX & MÜLLER (1975) separated *Rhytid-*

hysteron and *Eutryblidiella* by ascribing terminal germ-pores to ascospores of *Eutryblidiella*. In our recent studies of the genus, we have found germ-pores lacking in *E. hysterina*; there are pores in the ascospores of an unrelated species, *E. sabina* (DE NOTARIS) HÖHNEL which PETRINI et al. (1979) placed in its own genus, *Holmiella* PETRINI, SAMUELS & E. MÜLLER.

Eutryblidiella hysterina is close to *R. rufulum*. The ascomata have the same morphology. Young ascomata of *E. hysterina* are hysteriform; they have a bright orange pseudoepithecium when open and are hysteriform, triradiate or triangular when dry. The pseudoepithecium in both species is formed by the disintegrating remains of the tips of the paraphyses which are embedded in a gelatinous matrix, and the paraphyses are enclosed in a gelatinous sheath that stains blue in Melzer's reagent. Anatomy of the two species is nearly identical. Like *R. rufulum*, *E. hysterina* has a *Diplodia*-like and an *Aposphaeria*-like anamorph (SHEAR 1933, BAUMEISTER 1957) and is found on recently killed or still living branches. *Eutryblidiella hysterina* is most frequently found in southern Europe on *Buxus* spp., although it has also been reported on *Ilex* sp. in the U.S.A. (SHEAR 1933) and, as *E. panchanani* MUKERJI & DHAWAN 1968 (=*E. hysterina*), on *Prosopis* sp. and *Diospyros* sp. in India.

There are only two major differences between *R. rufulum* and *E. hysterina*. Ascospores of *E. hysterina* have one septum whereas those of *R. rufulum* have three; and paraphysoidal elements arise from the hymenium of the *Diplodia*-like phase of *E. hysterina* whereas such elements are not formed by *R. rufulum*. The similarities between these species exceed their differences, and we propose the transfer of *E. hysterina* to the older genus, *Rhytidhysteron*.

Of the seven species included in *Eutryblidiella* most are unrelated to the type species or to each other (PETRINI & al. 1979, HAFELLNER 1979). Only *E. panchanani* is congeneric with *E. hysterina* and we consider the two species to be synonymous. The holotype and paratype specimens of *E. panchanani* differ from typical *E. hysterina* only in having black pseudoepithecia and a negative reaction to Melzer's reagent. Ascospores are the same size and ascomatal anatomy is the same. Pseudoepithecial color cannot be used to delimit species within this group, as was seen with *Rhytidhysteron rufulum*, and it is now clear that the reaction to iodine may be variable within a species (KOHN & KORF 1975, NANNFELDT 1976, ROGERS 1979). A *Diplodia*-like stage was found on the type specimen of *E. panchanani* but it could not be distinguished from the *Diplodia*-like anamorph of *E. hysterina*. With only two specimens upon which to base our conclusions, we cannot accurately judge variation within *E. panchanani* but the information we have at present does not support its retention as a distinct species.

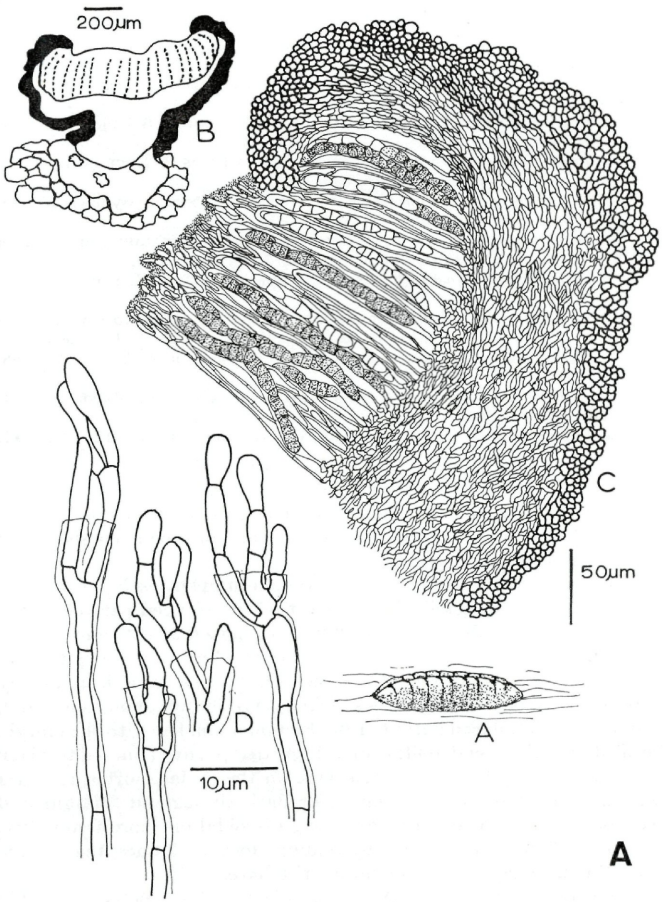

Fig. A. *Rhytidhysteron rufulum*. — A. Habit sketch of a young ascoma. — B. Longitudinal section of an open ascoma, diagrammatic. — D. Paraphyses showing the gelatinous sheath

Description of species

1. *Rhytidhysteron rufulum* (SPRENGEL ex FRIES) SPEGAZZINI, An. Soc. Cient. Argent. 90: 177. 1920. Fig. A: A—D; Fig. B: A—G

≡ *Hysterium rufulum* SPRENGEL, Kongl. Vetensk. Acad. Handl. 1820: 50. 1820.

≡ *Hysterium rufulum* SPRENGEL ex FRIES, Systema Mycologicum 2: 584. 1823.

≡ *Tryblidiella rufula* (SPRENGEL ex FRIES) SACCARDO, Sylloge Fungorum 2: 757. 13 Jun 1883.

≡ *Tryblidium rufulum* (SPRENGEL ex FRIES) ELLIS & EVERHART, North American Pyrenomycetes p. 690. 1892.

≡ *Rhytidhysteron rufulum* (SPRENGEL ex FRIES) PETRAK, Sydowia 5: 185. 20 Dec 1951. [as *Rhytidhysterium*]

= *Tryblidium rufulum* (SPRENGEL ex FRIES) SACCARDO var. fuscum ELLIS & EVERHART, Jour. Mycol. 5: 30. Mar. 1889.

≡ *Tryblidiella fusca* (ELLIS & EVERHART) REHM, Hedwigia 39: 83. 26 Feb 1900.

≡ *Tryblidiella rufula* (SPRENGEL ex FRIES) SACCARDO var. *fusca* (ELLIS & EVERHART) REHM, Ann. Mycol. 2: 524. 10 Dec 1904.

= *Rhytidhysteron brasiliense* SPEGAZZINI, An. Soc. Cienc. Argent. 12: 188. 1881.

≡ *Tryblidiella brasiliense* (SPEGAZZINI) REHM, Ann. Mycol. 2: 524. 10 Dec 1904.

= *Rhytidhysteron viride* SPEGAZZINI, An. Soc. Cienc. Argent. 12: 188. 1881.

ANAMORPHS. — *Diplodia*-like and *Aposphaeria*-like.

TELEMORPH. — Stroma. Subcortical, poorly developed, visible as a thin, spreading, black layer on surface of wood, consisting of brown, thin-walled, 4 µm wide hyphae.

Ascomatal morphology. Ascomata erumpent, solitary or cespitose; when young sessile, linear, most often arranged parallel to the long axis of the substrate, 1.5—2.0 mm long × 0.5—1.0 mm wide × 0.5—1.0 mm high in the middle, with acute ends and a deep, longitudinal slit extending the entire length of the ascoma, and with deep, evenly spaced, transverse costae or less often smooth; becoming discoidal, circular to lenticular in outline, 1.0—1.5 mm diam, flat with the margin inrolled over the pseudoepithecium. Pseudoepithecium red to black when fresh and when dry; when dark on the surface, often red in a zone adjancent to the hymenium, or dark throughout. Margin and receptacle black. When dry, remaining discoidal or, more frequently, the edges of the disc reclosing by folding along 1—3 lines, the ascoma becoming hysteriform, triangular or triradiate.

Ascomatal anatomy. Asci bitunicate, 6—8-spored, 77—100 (—112) × 9—12 (—17) µm, clavate to broadly cylindrical, tapering gradually to the pedicellate base, apex with a pronounced "nasse apicale"; contents bright orange in Melzer's reagent, orange coloration fading in chloral hydrate; ascospores uniseriate with overlapping ends and filling the entire ascus. Ascospores (19—) 26—36 (—43) ×

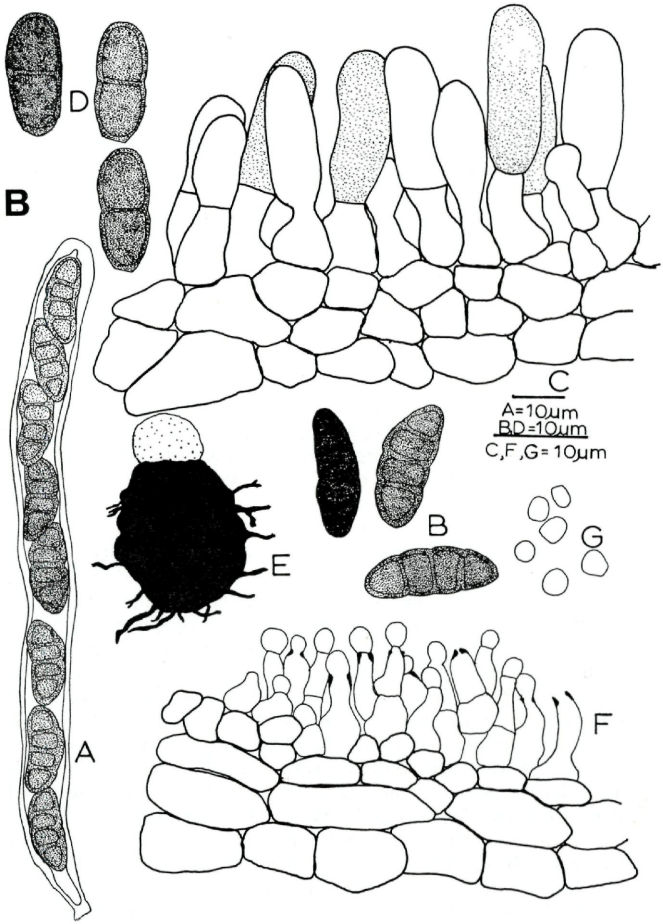

Fig. B. *Rhytidhysteron rufulum*. — A. Ascus. — B. Ascospores. — C. Portion of the lower wall of a *Diplodia*-like pycnidium. — D. *Diplodia*-like conidia. — E. Sketch of *Aposphaeria*-like pycnidium. — F. Portion of the lower wall of an *Aposphaeria*-like pycnidium. — G. *Aposphaeria*-like conidia

3.5—4.5 (—6.5) µm, 3-septate, reniform to elliptic to fusiform with rounded to subacute ends, at most slightly constricted at the septa, brown and translucent to nearly black and opaque; wall 1—1.5 µm thick, composed of two layers, smooth. Paraphyses exceeding the ascus by 15—25 µm, branched dichotomously immediately below the tip, terminal cells short, 7—10 µm long and clavate tapering gently from 2.5—3.5 µm wide at the apex to 1.5—2 µm basally, disintegrating and embedded in an amorphous substance to form the 40—50 µm thick pseudoepithecium; a zone 25—35 µm wide immediately above the ascal apices becoming blue-green in Melzer's reagent, blue coloration fading slowly in 100% lactic acid and rapidly in 3% KOH, reaction reversible; paraphyses branching sparingly along their length, enclosed in a gelatinous sheath which becomes blue-green in Melzer's reagent, the reaction apparently identical to that produced in the pseudoepithecium; paraphyses adherent to each other in 100% lactic acid and tightly enclosing the asci; asci coming free from this plexus in water. Subhymenium 20—25 µm thick, hyaline, red in Melzer's reagent and becoming colorless in 3% KOH and 100% lactic acid reaction reversible; consisting of tightly interwoven, horizontally oriented, short hyphae (the tissue appearing prosenchymatous), with slightly thickened walls, merging below with the medullary excipulum; terminating at the base of the margin. Medullary excipulum tightly compact, extending from the subhymenium to the base ascomatal, hyaline; becoming red in Melzer's reagent, color fading in 100% lactic acid, reaction reversible; consisting of basically vertically oriented, interwoven, hyphal cells 10—20 µm long × 5—8 µm wide with walls ca. 2 µm thick, cells at the exterior rectangular to elliptic in outline, vertically oriented, 20—40 × 10—15 µm and darkly pigmented at the outside where they form the flanks of the ascoma; not possible to distinguish an ectal excipulum; tissue of the medullary excipulum continuous with the margin. Margin tapering gradually from ca. 50 µm wide at the top to ca. 100 µm wide at the base, inner 25—50 µm consisting of hyaline, compact, interwoven hyphal cells, 10—17 µm long × 3—5 µm wide with walls 1—1.5 µm thick, merging below with the medullary excipulum; outer 25—50 µm consisting of cells nearly circular in outline, 7—10 µm in diam with walls 1—1.5 µm thick, heavily pigmented, pigmented at the exterior in cell walls as well as appearing as a black, amorphous accretion on the surface of the cells; merging below with external cells of the flanks.

CHARACTERISTICS IN CULTURE. — Ascospores germinating within 12 hrs on malt extract agar (2% malt extract, 0.5% yeast extract) at ca. 20 C, a single, straight, unbranched, germ-tube 200 µm in length arising from one end of each ascospore. Colonies spreading rapidly on malt extract agar and potato dextrose agar (Difco), when grown under a mixture of "cool white" fluorescent and near-UV light,

12 hr light/12 hr darkness, at 18—20 C, olivaceous with some red-brown aerial hyphae, cottony. Pycnidia forming within 3 weeks on potato dextrose agar.

Diplodia-like pycnidia abundant to rare, immersed, non-stromatic, subglobose, 460 µm high × 400 µm wide, papillate or non-papillate, or seated on the surface of the agar and pyriform, ca. 270 µm high × 130—180 µm wide and with a papilla 130—180 µm long × ca. 70 µm basally, black, smooth; pycnidial wall ca. 45 µm wide, consisting of pseudoparenchymatous cells 8—20 × 8—10 µm, thin-walled, brown; conidiogenous cells forming in a single layer over the entire inner surface of the pycnidial wall, consisting of a hyaline, globose cell 4—5 µm in diam basally and with a ca. 5 µm long elongation. Conidia arising holoblastically from the tip of the elongation of the conidiogenous cell, at first hyaline and unicellular, becoming dark brown to opaque and 1-septate with a pore in the middle of the septum following discharge, oblong with a truncate, non-cicatrized base, (17.5—) 19.5—23.5 (—29.5) × (6.5—) 8—10 (—12) µm, smooth.

Aposphaeria-like pycnidia forming abundantly in aerial mycelium, often associated with small tufts of red-brown hyphae, non-stromatic, globose to oblong, 100—150 µm high × 70—150 µm wide, non-papillate, black; phialides forming in a single layer over the entire inner surface of the pycnidial wall, hyaline, ampulliform to cylindrical, 4.5—9.0 µm long × 1.5—3.0 µm diam basally, and ca. 1.5 µm wide at the opening; collarette only slightly thickened, not flared. Conidia globose to elliptic, 2—3 µm diam or ca. 3.0 × 2.5 µm, smooth, held in a drop of hyaline slime at the pycnidial opening.

HABITAT. — On wood of a wide variety of living or dead, dicotyledonous plants.

DISTRIBUTION. — World wide in warm tropical and subtropical climates.

SPECIMENS EXAMINED. — Argentina (3) [1]), Bahamas (2), Bermuda (2), Bolivia (1), Brazil (30), Chile (1), Colombia (2), Cook Islands (2), Costa Rica (4), Cuba (6), Dominican Republic (1), Haiti (1), India (4), Jamaica (4), Java (1), Mexico (2), Puerto Rico (24), St. Croix (1), St. Thomas (3), Tonga (2), Trinidad (5); U.S.A.: Alabama (1), Florida (10), Louisiana (1), Ohio (3), South Carolina (1), Texas (1); Venezuela (1).

The above specimens include: Brazil: Faxina J. Puiggari 1230, Jun 1880 (LPS: HOLOTYPE *R. viride*). — Iguapé, rotten wood, J. Puiggari 1444, 10 Oct 1877 (LPS: HOLOTYPE *R. brasiliense*). — U.S.A.: Florida, near Jacksonville, on dead limbs, leg. W. W. Calkins Nr. 1013, Jan 1889 (NY, HOLOTYPE *Tryblidium rufulum* var.

[1]) The numbers in parentheses are the numbers of specimens examined from the areas. The specimens are deposited in LPS, NY, PDD or ZT.

fuscum). — Florida, Gainesville, on *Pistacia chinensis*, leg. R. K. Vorhees, 18 Dec 1934; same data, Vorhees Nrs. 12166, 12167, 12169. 15 Oct 1937 (NY).

The following collections were grown in pure culture: Brazil: Territorio de Roraima, along the Manaus-Caracarai Rd. at a point ca. 333 km from the intersection of the Manaus-Itacoatiara Rd., on wood, Dumont (BR 431), Hosford, Samuels, Buck, Araujo, Souza & Bernardi, 17 Nov 1977 (NY, INPA). — Amazonas, white sand Igapo, N. of Manaus, on wood, Samuels, Keel & Guitez, 14 Dec 1977 (Dumont-BR 997: NY, INPA). Chile: Parque Botanico Hualpen, on wood of *Peumus boldus* Molina, Oehrens & Garido comm. H. Butin, 24 Sep 1978 (PDD 39479). — Colombia: Dpto. Magdalena, Sierra Nevada de Santa Marta, between Palo Alto (1700 m) and Refugio de la Sierra (1850 m), on indet. branch. Dumont (CO 9000), Ryvarden, Oberwinkler, Buriticá, Pulido & Aquirre, 19 Jun 1978 (NY).

NOTES. — Isotypes of *Tryblidium rufulum* var. *fuscum* were distributed by Ellis and Everhart in North American Fungi 2331. Two portions of this essiccata in NY are *Hysterographium mori* (Schweinitz) Rehm; there are no ascomata of *Rhytidhysteron rufulum* on either portion. The holotype of this variety, cited above, is *R. rufulum*.

The specimen of *R. viride* was immature and had no ascospores. The ascomata are unexpanded; when sectioned the pseudoepithecium is entirely black. The type specimen of *R. brasiliense* contained only one old and well westhered ascoma. We did not make a microscopic preparation of it. In their external morphologies, *R. viride* and *R. brasiliense* are *R. rufulum*.

A red coloration is often present in wood. This coloration was also observed with *R. hysterinum*. It is apparently related to the presence of the fungus since no pigmentation in the wood was observed apart from the ascomata.

Red pseudoepithecial color is often associated with a red pigment that is soluble in3% KOH. Pseudoepithecia that are dark throughout often release a pale green pigment when immersed in 3% KOH. Red pseudoepithecial forms never released a green pigment and a red pigment never came out of a dark pseudoepithecial form; often no pigment came out of the pseudoepithecium regardless of its color.

2. *Rhytidhysteron hysterinum* (J.-M. L. Dufour) Samuels & E. Müller, comb. nov. Fig. C: A—B; Fig. D: A—F

≡ *Tryblidium hysterinum* J.-M. L. Dufour, Ann. Sci. Nat. 13: 321. 1828.
≡ *Tryblidiella hysterina* (J.-M. L. Dufour) Shear, Mycologia 25: 278. 1 Aug 1933.
≡ *Eutryblidiella hysterina* (J.-M. L. Dufour) Petrak, Sydowia 13: 242. 5 Nov 1959.

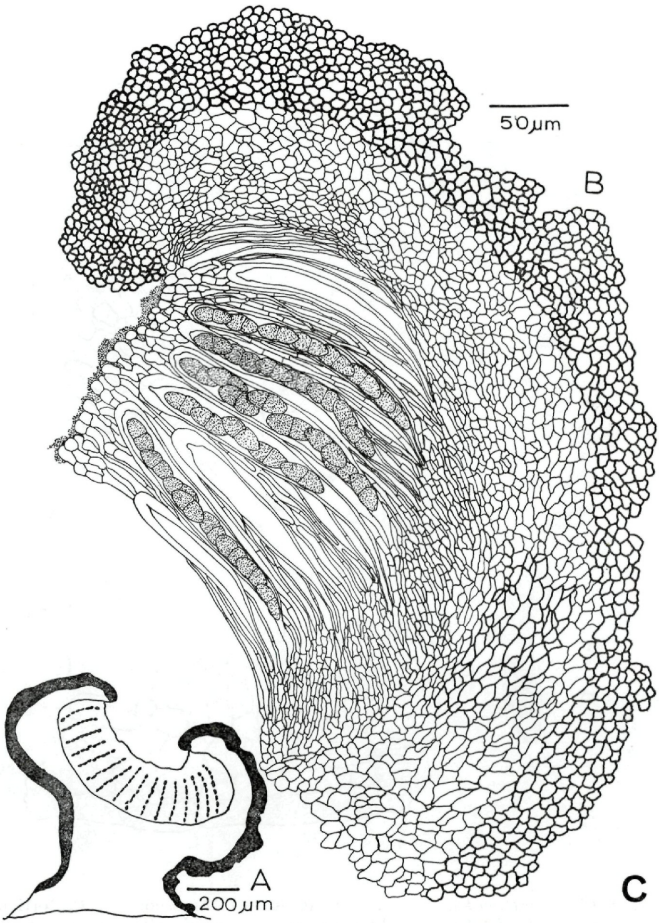

Fig. C. *Rhytidhysteron hysterinum.* — A. Longitudinal section of an open ascoma, diagrammatic. — B. Longitudinal section of the margin of a mature ascoma

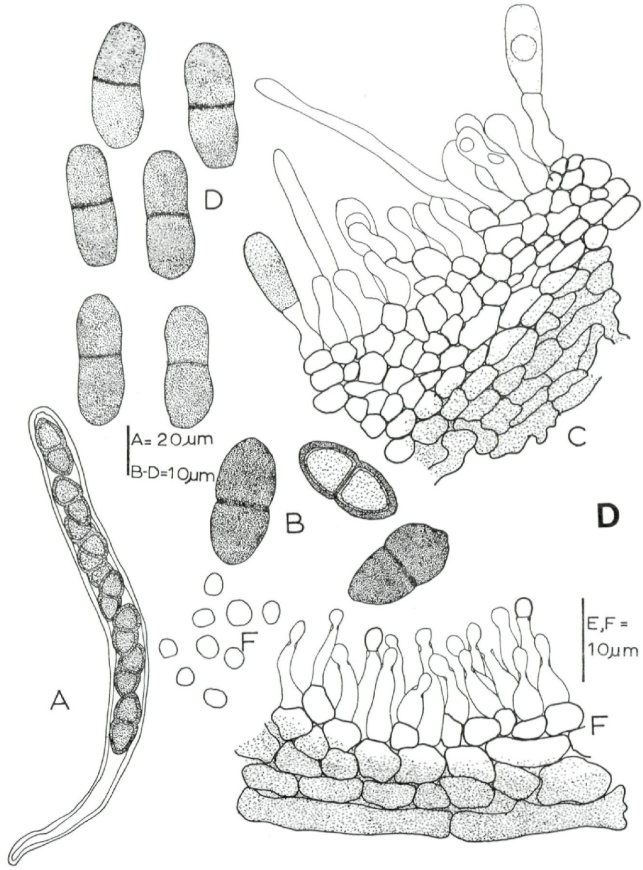

Fig. D. *Rhytidhysteron hysterinum*. — A. Ascus. — B. Ascospores. — C. Portion of the lower wall of a *Diplodia*-like pycnidium. — D. *Diplodia*-like conidia. — E. Portion of the lower wall of an *Aposphaeria*-like pycnidium. — F. *Aposphaeria*-like conidia

= *Hysterium elevatum* PERSOON ex DUBY in Botanicon Gallicum 2 ed., 2: 719. 1830.
≡ *Hysterographium elevatum* (PERSOON ex DUBY) DESMAZIERES, Ann. Sci. Nat. Bot. 3 sér., 20: 230. 1853.
≡ *Tryblidiella elevata* (PERSOON ex DUBY) REHM in Rabenhorst's Kryptogamen. — Fl. 2 Aufl., 1 Pilze III Abt., Ascomyceten p. 233. 1896.
= *Eutryblidiella panchanani* MUKERJI & DHAWAN, Nova Hedwigia 16: 433. 1968 (1969).

ANAMORPHS. — *Diplodia*-like and *Aposphaeria*-like.

TELEOMORPH. — Stroma. Subcortical, poorly developed, visible as a thin, spreading, black layer on surface of wood, consisting of brown, thin-walled hyphae.

Ascomatal morphology. Ascomata erumpent, solitary or cespitose, sessile; when young linear, most often arranged parallel to the long axis of the substrate, 1—2 (—3) mm long × 0.5mm wide × 0.5—1.0 mm high in the middle with acute ends and a deep longitudinal slit extending the entire length of the ascoma and with irregularly spaced, transversally arranged cracks; becoming discoidal, circular to lenticular in outline, 1.0—1.5 mm diam, flat, with the margin inrolled over the pseudoepithecium. Pseudoepithecium organe or black when fresh and when dry. Margin and receptacle black. When dry remaining discoidal or, more frequently, the edges of the disc reclosing by folding along from 1—3 lines, the ascoma becoming hysteriform, triangular or triradiate.

Ascomatal anatomy. Asci bitunicate, 4—8-spored, (170—) 185—220 (—235) × (13—) 15—17 (—20) µm, cylindrical, the lower (20—) 30—50 (—90) µm of each ascus devoid of ascospores, base pointed to pedicellate, apex with a pronounced "nasse apicale", contents bright orange in Melzer's reagent; ascospores uniseriate with overlapping ends. Ascospores (21—) 23—31 (—32) × (8—) 9—11 (—12) µm, 1-septate, septum median, fusiform with rounded to acute ends, slightly constricted at the septum, brown and translucent to nearly black and opaque with septum obscured; wall 1—1.5 µm thick, composed of two layers, smooth. Paraphyses exceeding the ascus by ca. 25 µm, branching dichotomously just below the tip; cells of the terminal 15—25 µm short, 4—7 µm long; tip cells globose to clavate, 3—5 µm diam, disintegrating and embedded in an amorphous substance to form the pseudoepithecium; a zone ca. 25 µm wide immediately above the ascal apices becoming blue-green in Melzer's reagent, blue coloration fading slowly in 100% lactic acid and rapidly in 3% KOH, reaction reversible; paraphyses branching sparingly along their length, enclosed in a gelatinous sheath which becomes blue-green in Melzer's reagent the reaction apparently identical to that produced in the pseudoepithecium; paraphyses adherent to each other in 100%

lactic acid and tightly enclosing the asci, asci coming free from this plexus in water: Subhymenium merging with the medullary excipulum, hyaline, red in Melzer's reagent and becoming colorless in 3% KOH and 100% lactic acid, reaction reversible, consisting of tightly interwoven, vertically oriented, short hyphae (the tissue appearing prosenchymatous), with slightly thickened walls, terminating at the base of the margin. Medullary excipulum tightly compact, extending from the subhymenium to the ascomatal base, hyaline to light brown, becoming red in Melzer's reagent, color fading in 100% lactic acid, reaction reversible; consisting of vertically oriented, interwoven, hyphal cells, 10—15 μm long × 5—8 μm wide with walls 1.0—1.5 μm thick, heavily pigmented at the exterior where the pigment is in the walls as well as being a black, amorphous accretion on the surface of the cells; cells toward the interior less heavily pigmented; merging below with cells of the flanks.

CHARACTERISTICS IN CULTURE. — Ascospores germinating within 12 hrs on malt extract agar (ME, 2% malt extract, 0.5% yeast extract) at ca. 20 C; a single, straight, unbranched germ-tube >200 μm long arising from each ascospore. In one week at ca. 20 C under a mixture of "cool white" fluorescent and near-UV light, 12 hrs light/12 hrs darkness, colonies on potato dextrose agar (PDA, Difco) ca. 1 cm diam, white and translucent, with submerged chains of swollen hyphal cells forming in the middle of the colony; within one month colonies on ME and PDA ca. 9 cm diam, flat, dark olivaceous, nearly black with scant, cottony, olivaceous aerial mycelium.

Diplodia-like pycnidia forming on ME within one month, immersed, non-stromatic, subglobose, ca. 350 μm high × ca. 400 μm wide, non-papillate, black, smooth. Pycnidial wall 25—35 μm wide, consisting of pseudoparenchymatous cells 5—7 × 3—4 μm, thin-walled, brown; conidiogenous cells forming in a single layer over the entire inner surface of the pycnidial wall, barely distinguishable from cells of the wall; consisting of a basal cell 6—7 μm across, and a 5—10 μm long elongation. Conidia arising holoblastically from the tip of the conidiogenous cell; at first hyaline and unicellular, becoming dark brown, opaque, minutely punctate and 1-septate with a pore in the middle of the septum, oblong, with a truncate, non-cicatrized base, (16—) 22—26 (—28) × (7—) 9—11 (—13) μm. Paraphysoids arising from among conidiogenous cells, up to 50 μm long × ca. 3 μm wide, septate, unbranched, with rounded ends, hyaline.

Aposphaeria-like pycnidia produced abundantly in the aerial mycelium and on the surface of the agar on both ME and PDA, non-stromatic, globose with a short papilla, 250—330 μm high × 220—250 μm wide, black; phialides forming in a single layer over the entire inner surface of the pycnidial wall, hyaline, ampulliform to cylindrical, 7—8 μm long × ca. 2 μm wide basally and ca. 1.5 μm wide at the

opening, collarette only slightly thickened and not flared. Conidia globose, 2—3.5 µm diam, smooth, held in hyaline slime at the pycnidial opening.

HABITAT. — On wood of *Buxus* spp., less frequently on *Ilex* sp., *Diospyros* sp. and *Prosopis* sp.

DISTRIBUTION. — World wide in warmer, northern hemisphere latidudes; not known from the southern hemisphere.

SPECIMENS EXAMINED. — FRANCE: Alpes Maritimes: Fontan (Royatal), Val Cairos, on *Buxus sempervirens* L., E. MÜLLER, 29. 6. 1961 (2 specimens, ZT). — Fontan, on *Buxus sempervirens*, E. MÜLLER, 26. 6. 1955 (ZT). — Pont du Loup, Branafan, on *Buxus sempervirens*, LOEFFLER, 21. 6. 1956. — Hautes Pyrenees: Luz, St. Sauvour, on *Buxus sempervirens*, F. CANDOUSSAU, 28 Jun 1978 (PDD 38822, Samuels culture 78—30). — Vaucluse, Col de Murs, *Buxus sempervirens*, E. MÜLLER, 30. 5. 1970 (ZT). — Forêt de St. Lambert, *Buxus sempervirens*, E. MÜLLER, 25. 5. 1962 (ZT). — INDIA: New Delhi, Mehrauli, *Diospyros cordifolia* ROXB., S. DHAWAN DU-K2S, 18. 10. 1960 (IMI 130289 and Herb. K. G. MUKERJI). ISOTYPES *Eutryblidiella panchanani*, Mehrauli, *Prosopis spicigera* L., S. DHAWAN, 8. 10. 1966 (IMI 130288).

NOTE. — All the French collections of *R. hysterinum* that we studied gave a positive reaction in the paraphyses to Melzer's reagent, while neither of the two Indian specimens of *E. panchanani* were iodine positive. It would be useful to examine additional specimens of *R. hysterïɑum* from other areas, to determine variability of the iodine reaction.

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