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New and rare fungi from cherry fruits

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Abstract: *Capronia hystrioides* sp. nov. (anamorph *Phaeoramularia hachijoensis*) was isolated from cherry fruit. Also isolated were *Sporormiella subticinensis* comb. nov., *Leptodiscella africana*, and *Cladosporium malorum*, a synonym of *C. porophorum*.

Key Words: *Capronia hystrioides, Cladosporium malorum, C. porophorum, Leptodiscella africana, Phaeoramularia hachijoensis, Prunus avium, Sporormia, Sporormiella subticinensis*

In the course of etiological studies of preharvest infections of cherry fruits (*Prunus avium* L. cv. Bing) we recovered several isolates which were undescribed, rare, or not previously reported from North America. As with the majority of strains from this study, these unusual fungi were isolated onto agar media from receptacular and/or stylar scars of surface-disinfested fruits collected prior to harvest (Dugan and Roberts, 1994).

One such isolate, ST10-7 (= ATCC 96019), consistently produced both teleomorph and anamorph on ½V-8 agar (90 ml V-8 juice, 3 g $CaCO_3$, 15 g agar, 910 ml H_2O) when exposed to a 12 h: 12 h light-darkness cycle of near ultraviolet plus fluorescent light at 20 C. Cultures derived from individually isolated asci were capable of producing pseudothecia. In culture, most asci in any given pseudothecium appeared aborted, but some asci produced viable ascospores. The fungus is provisionally placed in the Herpotrichiellaceae.

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Capronia hystrioides F.M. Dugan, R.G. Roberts et R.T. Hanlin, sp. nov. FIGS. 1–7

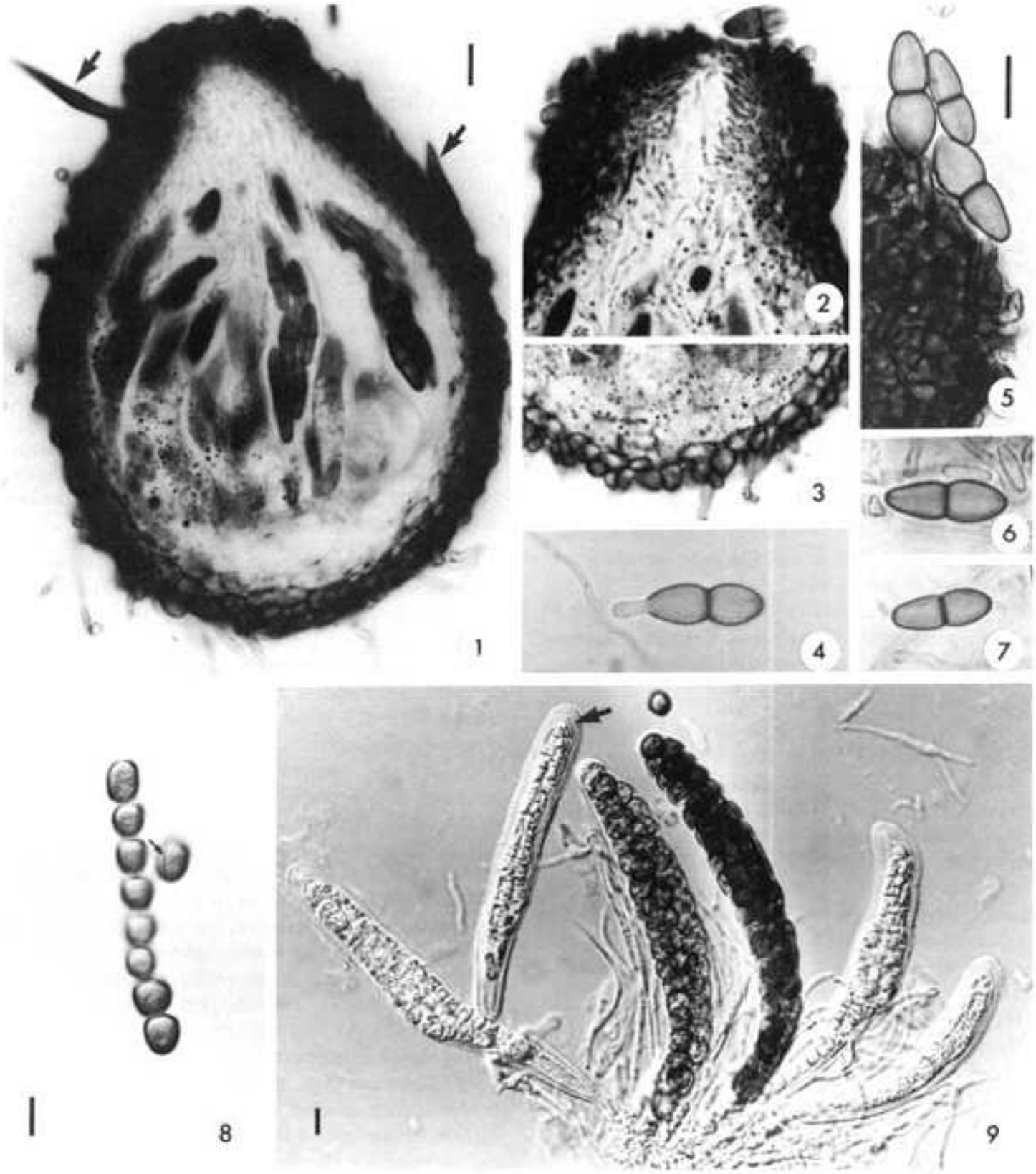
Pseudothecia nonstromatica, brunnea, superficialia vel immersa, setosa ex base ad papillam, papillata vel usque obpyriforma, 190–400 × 130–300 μm. Asci cum parietes crassis, 70–98 μm × 12–16 μm, cum pseudoparaphysibus. Ascosporae ellipticae, 1-septatae in mediana, ± constrictae ad septae, olivaceae, laevae, (15–)17–22 × 5.9–7.5 μm. Anamorphosis: *Phaeoramularia hachijoensis* Matsushima (FIG. 10).

HOLOTYPE. UNITED STATES. WASHINGTON STATE: Wenatchee, isolated from receptacular scar of cherry fruit, 7 July 1992, *F.M. Dugan and R.G. Roberts, ST10-7 (permanent slide, WSP 69609)*.

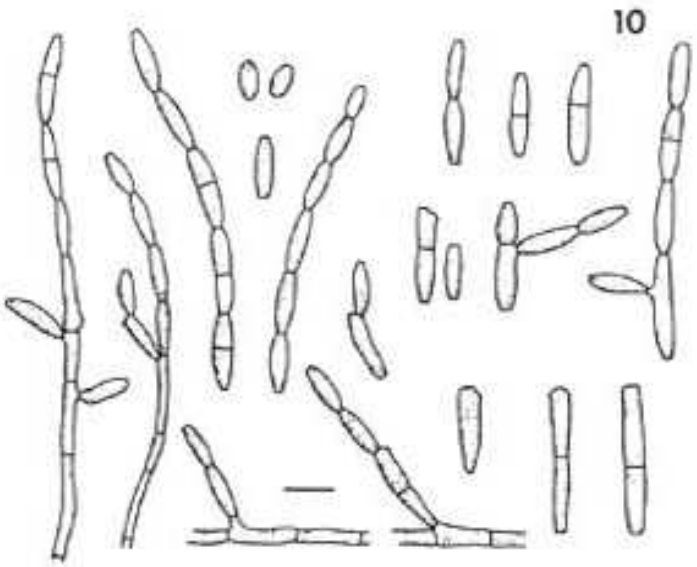
Pseudothecia nonstromatic (unilocular), brown, superficial to immersed, setose from base to papilla, papillate to almost obpyriform, 190–400 μm high, 130–300 μm wide. Asci thick-walled, 70–98 × 12–16 μm, with pseudoparaphyses. Ascospores elliptical, one-septate in the middle, ± constricted at the septa, olive, smooth, (15–)17–22 × 5.9–7.5 μm. Some older ascospores longitudinally striate.

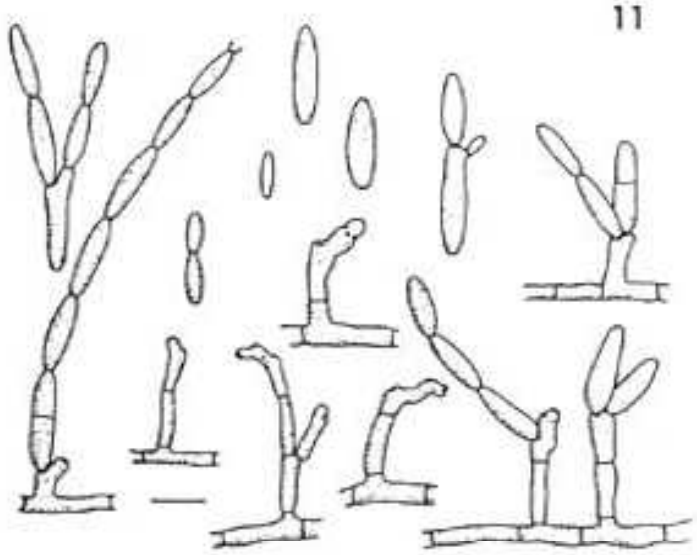
The specific epithet *hystrioides*, derived from *Hystrix* (a genus of porcupine), refers to the spinose nature of the pseudothecia.

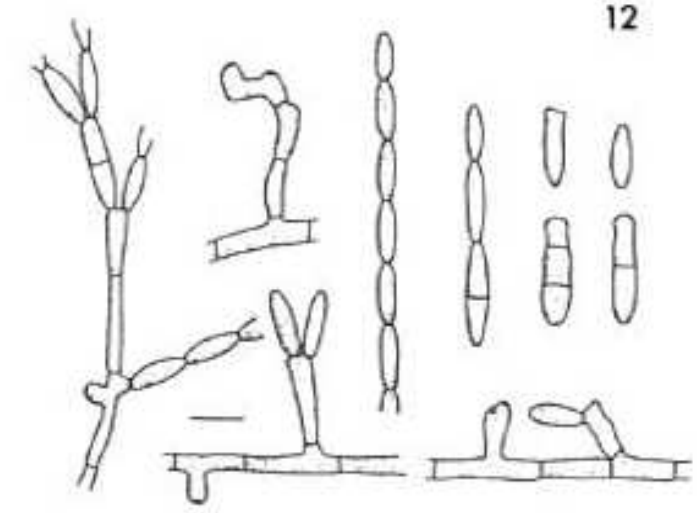
The olive color of the ascospores of ST10-7, the presence of pseudoparaphyses, and a *Cladosporium*-like anamorph might seem to indicate placement in the Venturiaceae. However, the pseudothecia of the Venturiaceae are commonly setose over the upper portion (as opposed to the entire fruiting body), and the ostioles do not contain periphyses (Barr, 1968, 1987). Because ST10-7 was often setose over the entire pseudothecium and because the ostiole contained short sterile filaments (FIG. 2), the teleomorph is probably better accommodated in the Herpotrichiellaceae. It should be remarked that the periphysoids of some members of the Herpotrichiellaceae have been noted to arise from the sides as well as upper portions of the locule, and to "serve as 'paraphyses' " (Barr, 1987). And although Müller et al. (1987) stated that sterile threads are not found among the asci of the Herpotrichiellaceae, Müller and von Arx (1962) explicitly stated the contrary for *Herpotrichiella*. Barr (1972) made reference to pseudoparaphyses and *Pleospora*-type development in *Dictyotrichiella*. The sterile threads in the upper portion of the locule of ST10-7 could be interpreted as periphysoids, in a manner analogous to



FIGS. 1–9. *Capronia hystrioides* ST10-7 (FIGS. 1–7) and *Sporormiella subticinensis* (FIGS. 8, 9). 1. Pseudothecium showing asci with ascospores, portions of setae (arrows) and pseudoparaphyses. 2. Portion of pseudothecium showing robust papilla, ostiole and pseudoparaphyses. 3. Section of lower pseudothecial wall. 4. Germinating ascospore. 5. Ascospores and wall fragment. 6. Ascospore and conidia. 7. Ascospore. 8, 9. *S. subticinensis*. 8. Ascospore and partspore with germ slit (arrow) (CV9-29). 9. Asci and pseudoparaphyses (CBS 124.66). Immature asci are conspicuously thick-walled (arrow). FIGS. 1–3, glycol methacrylate at 8–10 μm, toluidine blue and basic fuchsin. Bar in FIG. 1 for FIGS. 1–3; in FIG. 5 for FIGS. 4–7. Bars = 10 μm.







FIGS. 10–12. Conidia and conidiophores. FIG. 10. *Phaeoramularia hachijoensis* ST10-7, conidia and conidiogenous denticles. 11, 12. *Cladosporium malorum*, conidia and conidiophores. FIG. 11. ATCC 36953. FIG. 12. CV7-82. Bars = 10 μm.

Barr (1987), but because these threads did penetrate between the asci, we prefer the term pseudoparaphyses. The variability of sterile threads in the Herpotrichiellaceae requires further study. It should be remarked that some mycologists provide for olive-colored (in addition to brown) ascospores (Munk, 1957; Barr, 1972) and *Cladosporium*-like (in addition to *Exophiala*-like) anamorphs (Barr, 1987; Müller et al., 1987) in this family.

Müller et al. (1987) recognized only *Capronia* and *Acanthostigmella* in the Herpotrichiellaceae. Barr (1987), acknowledged the opinions of Müller et al., but retained polyspory and septation for generic characters. Eriksson and Hawksworth (1990) presented a taxonomic outline for the family that was largely congruent with the conclusions of Müller et al. (1987), and Barr (1991) abandoned polyspory as a generic character. We provisionally place the teleomorph of ST10-7 in *Capronia*.

The anamorph was as described and illustrated for *Phaeoramularia hachijoensis* (Matsushima, 1975) except conidia of ST10-7 were predominantly nonseptate instead of predominantly one-septate and slightly shorter than conidia of Matsushima's description. Conidia of ST10-7 were 0- to 1(2)-septate, olive, (4.5–)7–18(–22) × 2.9–3.5(–4.5) μm. Ramoconidia were 0- to 3-septate, 15–35 × 3–5 μm. Because Matsushima's type material was unavailable, we compared ST10-7 to Centraalbureau voor Schimmelcultures (CBS) isolate *P. hachijoensis* 462.82. When both strains were plated onto ½V-8 agar, our fungus grew an average of 30 mm in 18 da, whereas CBS 462.82 grew only an average of 5 mm. Conidia of CBS 462.82 were (6–)9–19(–26) × 2–3 μm. Ramoconidia were 0- to 3-septate, 18–30 × 2.5–3.5 μm. When 50 conidia of each fungus were measured and results analyzed with ANOVA by Systat Version 5 (Systat, Evanston, Illinois), a mean length of 14.1 μm for CBS 462.82 differed ($P = 0.001$) from a mean of 10.8 μm for ST10-7; a mean width of 3.1 μm for ST10-7 differed ($P = 0.001$) from a mean width of 2.5 μm for CBS 462.82. Although the conidia of CBS 462.82 were somewhat more frequently septate than those of ST10-7, most were nonseptate and the difference was not statistically significant. On some hyphae, conidiogenous denticles of CBS 462.82 tended to be longer with a more constricted apex than those of ST10-7; ST10-7 sporulated more profusely than CBS 462.82. On ½V-8 agar the colonies of CBS 462.82 were dirty yellow-brown (5E4) (Kornerup and Wanscher, 1978) with darker olive (5F4), compact margins; the colony of ST10-7 was olive-brown (4F4-5F4), with more diffuse margins. Under bright field microscopy both possessed olive hyphae, conidia and ramoconidia, but those of CBS 462.82 were slightly more brownish than those of ST10-7, and conidia

TABLE I. Means of ascus length and width (μm), ascospore length and width (μm), ranges for pseudothecial diameter (μm), and means for colony diameter (mm) on ½V-8 agar for *Sporormiella* isolates

Isolate	Ascus means	Spore means	Pseudothecia	Colony mean
CBS 124.66	163 × 25	72.1 × 10.5	270–400	46[a], 59[b]
CBS 125.66	167 × 27	76.4 × 10.5	160–400	52, 54
CV4-4a	156 × 26	76.9 × 10.6	150–540	40, 49
CV9-29	157 × 25	73.7 × 10.6	160–320	43, 61
CV9-84	159 × 25	71.5 × 10.2	290–400	50, 64

[a] Twenty-three days at 27 C.
[b] Thirty-three days at 20 C.

and ramoconidia slightly more hyaline. Thus ST10-7 and CBS 462.82 are distinguishable, but share the essential characters attributed to *P. hachijoensis* by Matsushima (1975).

The anamorph of *C. hystrioides* also resembled the illustration for *Stenella doliiforma* Matsushima (Matsushima, 1983), except that very few conidia of the latter fungus are pictured with a septum, and conidia of *S. doliiforma* are described as lighter (subhyaline) and slightly smaller than are those of our isolate or *P. hachijoensis*. Conidia of *P. hachijoensis*, and *S. doliiforma* were illustrated by Matsushima (1975, 1983) as borne on short denticles on the vegetative hyphae. Because *Phaeoramularia* Muntañola and *Stenella* H. Sydow are normally considered as possessing developed conidiophores, and because of the obvious similarity between Matsushima's two illustrations, the disposition of these species may change to reflect their similarity to each other and differences with other members of the above genera. *Phaeoramularia hachijoensis* belongs to a complex of fungi undergoing taxonomic and nomenclatural revision (Braun and Feiler, in press).

Because ST10-7 is greatly similar to CBS 462.82, and to Matsushima's illustration and description of *P. hachijoensis*, in construction of conidiogenous loci, conidial shape, conidial color, and colony color, we consider the anamorph of *C. hystrioides* to be *P. hachijoensis* in spite of the above differences with CBS 462.82 and in spite of some similarities with *S. doliiforma*.

Several isolates closely matching the description provided for *Sporormia subticinensis* Mout. (von Arx and Storm, 1967) were also recovered from cherry fruits. It has not been previously reported from North America. Three such isolates (CV4-4a, CV9-29, CV9-84) were compared to isolates of *Sporormia subticinensis* (CBS 124.66, CBS 125.66) that had been isolated from soil in Germany. On ½V-8 agar at 27 C and under lights as described above, our isolates produced colonies averaging 40–50 mm diam at 23 da; CBS isolates produced colonies averaging 46–52 mm. At 20 C, our isolates produced mean diameters of 49–64 mm; CBS isolates averaged 54–59 mm (TABLE I). All colonies on ½V-8 possessed grayish ruby to plum (approx 12E5) coloration throughout, with a thinly floccose, olive-grey overlay on centers and pale, narrow, concentric rings. On oatmeal agar (OA) (Stevens, 1974) under lights as above at 20 C, our isolates averaged 32–39 mm diam; CBS isolates averaged 36–39 mm. All colonies on OA were dark olive to olive-grey (approx 1F4), velutinus to tomentose, with broad concentric rings.

All isolates produced pseudothecia on ½V-8 agar within 55 da. Pseudothecia of all isolates were dark brown, subspherical to slightly papillate, with ostioles indistinct to lacking. With the exception of CV4-4a, which produced some larger pseudothecia, the collective size ranges of American isolates approximated those of the European isolates (TABLE I). When 20 asci of each isolate were measured and the results (TABLE I) analyzed by ANOVA, there were no significant differences overall ($P = 0.056$) for ascus length, but with pairwise comparisons (Fisher's LSD) the two CBS isolates differed ($P = 0.042$) from one another and CV4-4a differed ($P = 0.006$) from CBS 125.66. Similarly, there were no overall differences ($P = 0.073$) regarding ascus width, but with pairwise comparisons the two CBS isolates differed from one another ($P = 0.021$) and CBS 125.66 differed from CV9-29 ($P = 0.026$) and CV9-84 ($P = 0.018$). Overall differences were highly significant ($P = 0.001$) regarding ascospore length, with the two CBS isolates differing from one another ($P = 0.001$), CBS 124.66 differing ($P = 0.001$) from CV4-4a, CBS 125.66 differing ($P = 0.039$) from CV9-29 and CV9-84 ($P = 0.001$), and CV9-29 differing from CV4-4a ($P = 0.013$). There were no overall or pairwise differences between any isolates with regard to ascospore width.

Given the great similarity of all isolates in colony morphology and color, and given that significant differences in microscopic characters occurred within German and American isolates as well as between them, we conclude that these differences are intraspecific. The partspores (eight per ascospore) of all isolates possessed oblique germ slits (FIG. 8). Germ slits on terminal partspores were sometimes nearly longitudinal, whereas those on the other partspores were

transversely oblique. Although a subtle sheath had previously been observed at the septa between partspores on our Wenatchee isolates, only sheath-like remnants were seen on spores in later studies. Asci (FIG. 9) are conspicuously thick-walled early, but not late, in development. Pseudoparaphyses are abundant. *Sporormia subticinensis* was recorded but not examined in a revision of *Sporormia* de Not. and *Sporormiella* Ell. & Everh. (Ahmed and Cain, 1972). Because the latter monograph, in combination with Cain (1961), constitutes a comprehensive treatment of the Sporormiaceae, we follow the generic concepts presented there. Because of the presence of germ slits, and because ostioles were present in a high proportion of fruiting bodies, *S. subticinensis* would be best accommodated in *Sporormiella*.

Sporormiella subticinensis (Mout.) F.M Dugan et R.G. Roberts, comb. nov. FIGS. 8–9

BASIONYM: *Sporormia subticinensis* Mout., Bull. Soc. Roy. Bot. Belgique 36: 14. 1897.

A similar fungus, *Ohleriella neomexicana* Earle, recorded from New Mexico, has larger pseudothecia (Barr, 1990). Barr discounted, on morphological criteria, the suggestion of Ahmed and Cain (1972) that *O. neomexicana* (which they discussed as *O. mexicana* Earle) is the same as *Sporormiella octomera* (Auersw.) Ahmed & Cain. *Sporormiella subticinensis* may be closely allied to *O. neomexicana*, however, as the latter grows on wood (Barr, 1990) and the former has now been recovered from cherries (Dugan and Roberts, 1994) and wood (von Arx and Storm, 1967), as well as from soil. Our Wenatchee isolates grew poorly on rabbit dung agar.

We also recovered several isolates of a fungus matching the illustration for *Cladosporium porophorum* Matsushima (Matsushima, 1975). We compared one such isolate (CV7-82) to *C. porophorum* CBS 173.80 and to *Cladosporium malorum* Ruehle ATCC 36953 by measuring growth rates on ½V-8 agar at 27 C and lighting as described above, and by measuring 50 conidia of each isolate and analyzing results by ANOVA. CV7-82 achieved mean diameters of 69–70 mm in 8 da and was olive (approx 1E8-2E8) and slightly flocculent; CBS 173.80 ranged from 55–62 mm and was similar in color and morphology to CV7-82; ATCC 36953 ranged from 67–70 mm and was pale olive-grey (approx 2D2) to pale olive brown (approx 4D3). Each mean conidial length (11.9 μm for CV7-82, 13.6 μm for ATCC 36953, 15.8 μm for CBS 173.80) differed ($P \leq 0.002$) from the others in pairwise comparisons (Fisher's LSD). Means for widths (3.5 μm, 3.6 μm, 3.4 μm, respectively) did not differ. Conidia were in every case catenulate, nonseptate, olive brown, with cicatrized, truncate ends, and were borne on short, 0- to 2(3)-septate, cicatrized conidiophores (FIGS. 11, 12). Although *C. malorum* was recorded as a pathogen of apple by Ruehle (1931), his isolate (ATCC 36953) sporulated poorly on agar media and produced no lesions upon inoculation into ripe apple (cv. Golden Delicious). CV7-82 produced lesions matching the small, brown, dry lesions described by Ruehle. When all three isolates were subsequently inoculated into ripe apples (cv. Fuji), ATCC 36953 again failed to produce lesions, but lesions produced by the other isolates corresponded with those described by Ruehle. We conclude that the above differences in growth rates and conidial length are reflective of intraspecific variation, and that *Cladosporium porophorum* and *C. malorum* are conspecific, the older and thus correct name being *C. malorum* Ruehle.

A single isolate (ATCC 96022) of *Leptodiscella africana* (Papendorf) Papendorf, was obtained from cherry fruit. The fungus has been found previously in Africa (Papendorf, 1967), Alabama (ATCC 38843), the Netherlands (CBS 461.77) and Costa Rica (Bills and Polishook, 1994); this report constitutes a new record for *Prunus* and Washington state.

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