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THE ELLIPSOID-SPORED SPECIES OF PULVINULA (PEZIZALES)

RICHARD P. KORF and WEN-YING ZHUANG Plant Pathology Herbarium, Cornell University Ithaca, NY 14853 USA

ABSTRACT

The genus Pulvinula Boud. consists primarily of spherical-spored species. A single ellipsoid-spored species, P. ovalispora Boud., has been described, and previously reported only from the 1912 type collection from Algiers (Boudier, 1917; Pfister, 1976). That type specimen has been reexamined, and is shown to be conspecific with three North American collections, with one from Jamaica, and with one from the Canary Islands, all collected by the senior author. We have made four ellipsoid-spored collections of Pulvinula in China, but these differ sufficiently from Boudier's species to require description as P. ascoboloides n. sp., with a cyanophilous sheath surrounding the ascospores. Some comments on peculiaritiess of Pulvinula are appended.

Spherical ascospores characterize sixteen of the seventeen species of Pulvinula Boudier (1885) accepted by Pfister (1976) in his synopsis of the genus, and the five named species and two named varieties (plus the two unnamed "taxonomic species") added since then (Donadini, 1975; Kaushal, 1982; Kaushal et al., 1982; Nemlich and Avizohar-Hershenzon, 1976; Svrcek, 1977; Schumacher, 1982; Waraitch, 1977). The genus is distinct from Lamprospora, in which many of the species had been earlier placed, in its gracile, delicate, usually hooked paraphyses [an exception is P. salmonicolor (Seav.) Pfister], and in general by possession of tapering ascus bases which, in many species, have a characteristic fork (cfr. Rifai, 1968). The sole ellipsoid-spored species to be referred to Pulvinula was P. ovalispora Boudier (1917), collected in Algiers in 1912, and apparently never since reported as having been collected again (Pfister, 1976).

The senior author collected an ellipsoid-spored **Pulvinula** in 1948 in Michigan, and submitted the material for determination to Dr Marcelle Le Gal, in Paris. In 1949 she confirmed the collection as **P. ovalispora**, based upon a

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comparison with the type specimen at PC. Since that time the senior author has collected the species four more times: twice in New York State, once in Jamaica, and once on the island of Tenerife, Canary Islands (close to the type locality, Algiers). In all cases the apothecia had abundant carotenoids, recorded as orange-salmon in fresh condition. One collection is marked "yellow," but whether that refers to fresh or dried material is not recorded. Dried apothecia of all collections are yellow.

In 1981 the senior author had the opportunity to collect discomvcetes in Sichuan Province, China, and he obtained two collections of an ellipsoid-spored Pulvinula on pebbles, soil, and plant debris that were greenish-yellow. He field-identified both collections as Ascobolus sp. The junior author also collected in Sichuan Province, in 1983, and also twice collected the same Ascobolus-like species. These four collections, though similar in many respects to P. ovalispora, differ markedly not only in apothecial color, but in ascospore size and, above all, in the presence of a well-developed, thickened, loosening, cyanophilic sheath around the ascospores (Fig. 1). This sheath is quite unlike any structure we know in other members of the Pezizales, (with the possible exception of the genus Jafneadelphus discussed below). The young ascospores of hoth ellipsoid-spored species have a thick, gelatinous wall (much as in Ascobolus and in Scutellinia), and are biguttulate in youth. As spore development proceeds, in both species, an evident cyanophilic spore wall is seen,, which at complete maturity becomes very thin, and is surrounded by another, thin, non-cyanophilic wall layer. The cytoplasm of the fully mature spore becomes refractive, as in the spherical-spored species. One, or sometimes two, at first spherical but eventually irregular, deBary bubbles (gas inclusions) can be seen in many such mature spores in P. ovalispora (Fig. o), but such dBbs appear to be rare in the Chinese species.

Through the kindness of Dr. J. Mouchacca (PC), we were able to examine the type specimen of **Pulvinula ovalispora**. It consisted of a single apothecium, glued to a slip of paper, together with a dried-down slide, apparently made by Mme Le Gal at the time she examined Boudier's type. The same specimen had been sent to Dr. Pfister when he was preparing his synopsis, but he apparently avoided making any mounts because of the fragmentary nature of the type specimen (Pfister, 1976). We debated amongst ourselves for some time, after examining Mme Le Gal's slide, whether we should make an additional mount. Her slide revealed ascospores, which we could measure, but few other anatomical features. We finally, reluctantly, soaked up the remaining apothecium (already partially dissected, probably by Mme Le Gal), and proceeded to section a portion of it on a freezing microtome, reglueing the remaining part on the original slip of paper. Our sections proved adequate to study the specimen and to reveal that it has essentially all of the characters displayed by the more recent and better-preserved collections. We have been unable to confirm the branching of the paraphyses illustrated in the sketch accompanying the type packet and also in Boudier's (1917) plate, either in the type material or in the recent collections. Some unmounted sections have been dried down on a slide and placed with the type specimen, and can be easily lifted off for future use by other investigators.

There are rather marked variations in spore and ascus measurements among the six collections of **P. ovalispora** (and even between the mount apparently made by Mme Le Gal and our sections from the same apothecium), but we consider these within the natural variation of the species (Figs. o, p). Professor Pfister (pers. comm.) would weigh ascus length heavily in **Pulvinula**, and if he is correct, assignment of CUP-MM 139 to **P. ovalispora** may be in error. Similar inter-collection differences were noted in the species from China. These data are summarized in Table 1.

TABLE 1. Measurements in micrometers of ellipsoid-spored Pulvinulae.

	Hymenium	Ascus	Spore Measurements		Min/Av/Max
Spec. #	Height	Width	Range	Average	1/w Ratios
			u la ovalispora Boud. re sheath absent)		
Type (PC) (old slide	?	?	12.4-16.8x6.5-10.3	13.6x7.2	1.4/1.9/2.1
Type (PC) (section)	140-160	9.0-10.8	11.0-15.4x5.9-8.0	12.6x6.7	1.6/1.9/2.2
RPK 1295	150-160	11.0-13.2	11.0-13.2x6.6-8.0	12.1x7.5	1.4/1.6/1.8
RPK 4065	ca.100	11.0-12.5	9.8-12.4x6.0-7.1	11.1x6.6	1.6/1.7/1.8
CUP 54657	ca.110	11.0-12.0	10.4-13.9x6.6-7.8	11.9x7.1	1.4/1.7/2.0
CUP-MJ					
401	120-130	11.0-14.5	11.0-13.2x6.6-7.6	12.3x7.2	1.5/1.7/1.9
CUP-MM					
139	250-260	10.2~11.7	13.2-16.1x7.7-11.0	14.3x8.9	1.4/1.6/2.1
			coboloides Korf & Zh ments exclude spore s		
CUP-CH					
2316	220-225	9.0-11.0	13.2-17.0×6.6-8.2	15.5x7.4	1.8/2.1/2.4
CUP-CH			1012 1.10A010 012	10.04/.4	****
2392	ca.240	9.5-10.2	14.0-18.0x7.1-8.8	16.1x7.7	1.9/2.1/2.4
HMAS					
45054	215-225	10.5-13.0	14.6-17.3x7.3-8.8	15.8x7.8	1.7/2.0/2.3
HMAS					
45055	230-260	9.5-12.4	14.0-17.6x7.3-8.1	15.8x7.7	1.8/2.0/2.2

The collection data and the range now known for Pulvinula ovalispora is as follows:

ALGERIA: PC-B (holotype). R. Maire #483. Koléa. Sur terre. 17.ii.1912.

CANARY ISLANDS (SPAIN): CUP-MM 139. R. P. Korf, W. C. Denison, L. M. Kohn & M. A. Sherwood. Just below west entrance to Monte de las Mercedes, Tenerife. On soil. 5.i.1976.

JAMAICA: CUP-MJ 401. R. P. Korf, J. R. Dixon, K. P. Dumont, R. W. Erb, D. H. Pfister, D. R. Reynolds, A. Y. Rossman & G. L. Samuels. Along Cane River and slope of Good Hope Mountain, near Kingston, St. Andrew Parish. On cow dung. 12.i.1971.

NEW YORK: R.P.K. 4065. R. P. Korf. Stone Quarry Road, Ithaca. On leaf of Populus sp. 30.v.1964.

CUP 54657. R. P. Korf & T. Plowman. Bergen Swamp, Genesee County. On dead leaves of Populus. 1.vii.1964.

MICHIGAN: <u>R.P.K.</u> 1295. **R. P. Korf.** A wooded hollow 1/10 mile S. of Eber White Woods, near Ann Arbor. On leaves. 1.vii.1948.

We present here the diagnosis of the new species:

PULVINULA ASCOBOLOIDES Korf & Zhuang, sp. nov. (Figs. a-n)

A Pulvinula ovalispora Boudier vagina ascosporica cyanophila differt.

Apothecium discoid, sessile, pale yellow, yellow, or greenish-yellow when fresh, 3-5 mm in diam, with a restricted subiculum around the base. Ectal excipulum of textura angularis, cells isodiametric, hyaline, $6.5-13.5 \mu m$ in diam; medullary excipulum of tightly interwoven, hyaline textura intricata, individual hyphae difficult to discern. Asci cylindrical, $180-250 \times 9.0-13.0 \mu m$, 8-spored, operculate, apex J-, thin-walled in youth and at maturity, but strikingly thick-walled after ascus elongation at about the time of ascospore delimitation, tapering often rather abruptly below to a stalk-like, cylindrical, often coiled or undulate, sometimes branching base. Ascospores hyaline, ellipsoid, smooth-walled, $13.2-18.0 \times 6.6-8.8 \mu m$, when first formed with a thick, gelatinous non-cyanophilic wall, soon with a strongly cyanophilic, much thinner wall, often biguttulate at that stage, at maturity with the innermost wall layer thin and cyanophilic and with a non-cyanophilic, somewhat thicker outer layer, all surrounded by a much thicker, irregular, loosening cyanophilic sheath that may collapse around all or part of the spore, at full maturity the spore cytoplasm distinctly refractive, guttules absent, deBary bubbles only rarely present. **Paraphyses** filiform, gracile, curved at the apex, septate, containing carotenoid pigments, branched at the base, ca. 1.5 µm wide.

Etymology: from the gross appearance of the apothecia, resembling a soil-inhabiting species of Ascobolus.

HOLOTYPE: CUP-CH 2316. R. P. Korf & R.-y. Zheng. Woods above Jianfugong, Qingchengshan, Guan Xian, Sichuan, China. On pebbles, soil, and duff. 15.ix.1981. ISOTYPE: HMAS 45098.

PARATYPES: CUP-CH 2392. R. P. Korf & R.-y. Zheng. Between Wudongtian and Chaoxiting, Qingchengshan, Guan Xian, Sichuan, China. On duff, leaves of various sorts, and Chamaecyparis leaves and fruits. 18.ix.1981. (= HMAS 45099)

HMAS 45054. W.-y. Zhuang. Emeishan, Sichuan, China. On soil. 25.vi.1983. (= CUP-CH 2480)

HMAS 45055. Same data. On pebbles. 25.vi.1983. (= CUP-CH 2481)

SOME THOUGHTS ON PECULIARITIES OF PULVINULA

The position of **Pulvinula** in the classification of Pezizales has generally been accepted as being close to **Lamprospora**, **Octospora**, **Byssonectria** and allied genera of the tribe Aleurieae, assigned to the family Pyronemataceae (Eckblad, 1968; Korf, 1972) or Pezizaceae sensu lato (Dennis, 1978). Certain features of the ellipsoid-spored, as well as the spherical-spored, species we have studied indicate to us that the genus may well deserve a more isolated position than it usually enjoys.

We call attention first to the peculiar ascus base of most species of the genus. The asci are usually borne from croziers that develop on remarkably thin hyphae, and the forked ascus base so evident in many species represents an almost diagnostic feature seldom encountered elsewhere in the Pezizales. Rifai (1968: figs. 225-228, 230, 232, 233) illustrates this feature very well. The base of the ascus is often rather abruptly narrowed to form a cylindrical tube that may become coiled or undulate, as in P. ascoboloides of this paper (Figs a-h) and in P. miltina (Berk.) Rifai as illustrated by Rifai (1968: figs. 224, 225). We find the free end of the crozier may even point upwards in our new species (Figs. f-h), not characteristic of the other species we know. The pig-tail like coiling of a cylindrical ascus base is characteristic of certain genera of the Sarcoscyphineae, to which Pulvinula shows little other resemblance.

The very thick-walled asci at the time of spore delimitation is another feature we find in the ellipsoid-spored species for which we have no clear counterpart in other members of the Aleurieae. The shift from thin-walled asci at formation (Fig. a), thick-walled (gelatinous walls?) at elongation (Fig. b), and thin-walled again at maturity (Fig. c) mimics the typical bitunicate ascus. If such ascus features are common in other members of this tribe, we have not seen mention of the phenomenon in the literature. Nor have we observed this feature in a brief survey of spherical-spored **Pulvinulae** in our collections at CUP.

The ascospores, too, seem anomalous in this tribe. In both spherical-spored and ellipsoid-spored **Pulvinulae** the ascospore takes on a refractive quality at maturity. Usually it is possible to see a deBary bubble in such ascospores when they are mounted in a medium of high osmotic concentration. The refractive quality resembles that found in the mature spores of **Fimaria** and some other members of the Pseudombrophileae. The ascospore contents must be nearly solid at maturity, since the deBary bubbles that form soon take on an irregular shape (Fig. o), and there may even appear to be more than one in a single ascospore, just as in **Coprobia**, a genus often assigned to the Scutellinieae.

Thick, gelatinous, non-cyanophilous ascospore walls in early youth (Figs. b, k) are characteristic of both the sphericalspored and the two ellipsoid-spored **Pulvinulae**, but can also be found in some species of **Scutellinia**, in **Coprobia**, and in a number of dung-inhabiting species and genera. Notable among these is, of course, **Ascobolus**, placed far distant in the Ascobolaceae. Since the senior author identified both of

Figs. a-o, Pulvinula spp. a-n, P. ascoboloides. a, base of a young, thin-walled ascus; b, base of an elongated, thick-walled ascus with one, young, thick-walled ascos spore shown; c, base of a mature ascus with two mature ascospores shown; d-i, ascus bases; j, apices of three paraphyses; k, three thick-walled, immature ascospores; l, mature ascospores with cyanophilic sheaths; m, two mature ascospores, probably slipped out of their sheaths; n, mature ascus with 8 ascospores. o-p, ascospores of P. ovalispora. (All figures x1000 except fig. n, x500. Figs. j and n from CUP-CH 2316; o, from CUP-MJ 401; p, from PC, section of Boudier type; all other figures from CUP-CH 2392.)



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his Chinese collections of the new species as **Ascobolus** while in the field, the general resemblance to that genus cannot be ignored.

Yet another Ascobolus-like feature of the new Chinese species is that the asci may possibly elongate to exceed the hymenium at the time of spore discharge. The senior author's field notes (hand lens) on one collection (CUP-CH 2316) state "with protruding asci dotting [hymenial] surface." We do not know if other species of **Pulvinula** share this character.

Perhaps the greatest problem we see is in the interpretation of the spore sheath in **P. ascoboloides.** A loosening perispore occurs regularly in **Cheilymenia** and in the closely related genus **Coprobia**, but that is a delicate structure quite unlike the thick, notably irregular, cyanophilic sheath in our fungus (Fig. 1). At spore maturity the sheath may loosen, and some smooth, sheathless spores can be seen outside asci (Fig. m), which may be spores which have slipped out of their sheaths. The sheath appears to be formed within the epiplasm, and it is tempting to compare it to the material that is deposited from the epiplasm onto the ascospore surface in **Ascobolus**. There, of course, the material deposited becomes pigmented, at first purple, soon brown (by oxidation?), and is closely appressed to the ascospore surface at maturity.

An entirely different spore sheath is that displayed by **Geneosperma** Rifai [a genus based on **Scutellinia geneospora** (Berk.) Kuntze], where the tapering ends of the sheath suggest that it is a membrane developed across the epiplasmic interfaces of the eight portions of cytoplasm each under ascosporic nuclear control.

Our studies of the members of the genus Jafneadelphus (Ascodesmidoideae in Korf's (1972) classification) have shown us a somewhat similar, thick, irregular, cyanophilous spore sheath surrounding the maturing ascospores. As in Pulvinula ascoboloides, the young ascospores are very thick-walled, and soon develop a thin cyanophilic wall, but in Jafneadelphus the sheath appears to become incorporated in the ascospore of most species of the genus. It seems that spore marking development in Jafneadelphus may more closely

approach the situation in Ascobolus than hitherto suggested, differing from the typical ornamentation of operculate discomycetes as described by Le Gal (1947). The mature ascospore in P. ascoboloides remains smooth, and there are no markings with which the sheath may combine. Except for the similar ascospore sheath, there is little to suggest any close relationship of Pulvinula with Jafneadelphus, however.

Given the unusual features of spherical-spored Pulvinulae, and the even more bizarre features of the ellipsoid-spored members, we agree with Pfister (1976) and feel that assignment to the Aleurieae is wholly unsatisfactory: "The species of the genus form a discrete unit which only superficially resemble other members of this tribe." We also hesitate, as did he, to erect a new taxon to accommodate the genus. To erect a new monogeneric family would perhaps best reflect merely our present lack of understanding of its closest relatives. A new monogeneric tribe within the Pyronemataceae would merely imply that the genus is isolated, without providing any clue to its real relationships. Further work on the carotenoid pigments may help, for Arpin (1968) discovered a unique monocyclic carotenoid in Pulvinula constellatio (Berk. & Br.) Boud., as already pointed out by Pfister (1976). For the present we will content ourselves with the mental note that Pulvinula is, indeed, peculiar.

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TWO NEW RUST FUNGI (UREDINALES)¹

GEORGE B. CUMMINS

Department of Plant Pathology, University of Arizona Tucson, AZ 85721

ABSTRACT

Cronartium arizonicum sp. nov. on Castilleja patriotica is the teliomorph with which Peridermium filamentosum Peck sensu stricto on Finus ponderosa belongs. The species is heteroecious and occurs in Arizona. Ravenelia sonorensis sp. nov. occurs on Acacia californica in Sonora, Mexico. It has peridiate aecia (Aecidium) and 2-celled central teliospores.

CRONARTIUM ARIZONICUM Cumm. sp. nov.

Status aeciosporiferis Peridermium filamentosum Peck in Pinus ponderosa Laws. Urediniosporae $(20-)22-26(-27) \times (13-)14-17(-18) \mu m$, obovoideae vel ellipsoideae, membrana 1-1.5 μm crassa, fere hyalina, echinulata, poris germinationis sparsis, obscuris. Teliae usque ad 1.5 mm longae, brunneae, sporae variabiles, plerumque 45-66 \times 9-12 μm , cylindraceae, membrana 0.5-1 μm crassa, flavida vel fere hyalina.

HOLOTYPE: on *Castilleja patriotica* Fern. near turnoff to Barfoot Park, Chiricahua Mts., near Portal, AZ, 10 Sept 1957, Cummins 57-307 (PUR 56446); isotypes issued in Solheim and Cummins Mycofl. Saximont. Exsic. No. 909 as *Cronartium coleosporioides* Arth.

This fungus often has been submerged in *Cronartium coleosporioides* Arth. but has dissimilar aecia and produces a quite different disease of pine. But the teliomorphic state that belongs with *Peridermium filamentosum* has had no name. *Cronartium arizonicum* fills this need. *P. filamentosum* is comprised of three races, two of which do not

P. filamentosum is comprised of three faces, and the third which infect Castilleja and apparently are autoecious, and the third which is heteroecious and occurs in southern Arizona (Peterson, Phytopathology 58:309-315. 1968.) This is C. arizonicum. Southern Arizona (the Santa Rita Mts.) also is the type locality of P. filamentosum. The precise distribution of C. arizonicum is not known but field observation indicates intimate association of aecial and telial states in areas south of the Grand Canyon.

¹University of Arizona Agricultural Experiment Station Journal article No. 3888.