ADDITION TO PHYLLOSTICTA PERSOON FROM PAKISTAN

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Abstract

History of *Phyllosticta* is traced and description of the genus is amended in the light of recent developments on conidiogenesis and appendage formation. A new species *Phyllosticta sultanii* and a new name *Phyllosticta ahmadii* are described, illustrated and compared with related taxa.

Introduction

The generic name *Phyllosticta* dates back from Persoon (1818), who introduced it for *Sphaeria lichenoides* DC., which orginally had two varieties var., *convallariae- polygonatae* DC., and var., *hederae-helicis* DC. Later De Candolle (1815) called the first variety *S. lichenoides* var., *convallariicola* DC., and this was renamed *Phyllostica convallariae* Persoon (=*P. convallariicola* Persoon) (1818). It serves as type species of the genus. According to Demazieres (1847), *Phyllosticta* is confined to leaves, with pycnidial conidiomata and hyaline, aseptate or uniseptate conidia. Saccardo (1878, 1884) referred to conidiomata as perithecia (pycnidia) occurring on leaves and with hyaline ovoid to oblong and aseptate conidia. This Saccardoan concept was so popular that *Phyllosticta* became restricted to species with small, hyaline, aseptate conidia formed in pycnidia on leaves. Van der Aa (1973) amended the classical concept of Saccardo and described 46 species of *Phyllosticta* in his monograph in which 4 new species, 5 new names and 12 new combinations were made. According to Van der Aa (1973), *Phyllosticta* is a pycnidial fungus occurring on leaves, fruits, stems or other parts of plants. Species are host specific at least at generic level.

Punithalingam (1974) studied the type of Phyllosticta convallariae Pers., and gave a detailed description based on the type which Van der Aa (1973) had not studied. According to Punithalingam (1974), P. convallariae Pers., is found on leaves. It produces oval to irregular lesions. Pycnidia are solitary, immersed, sphaerical, 150-200 µm, ostiolate, wall 4-6 cells thick, differentiated into an outer dark, thicker layer and a hyaline thinner inner layer. Conidiophores absent. Conidiogenous cells cylindrical and hyaline. Conidia aseptate, hyaline, subovoid with a truncate base 15-18 x 7-8 μ m, guttulate with an apical appendage 10-14 x 0.5 μm. Van der Aa (1973) did not describe the conidiogenous cells in *Phyllosticta* spp., however his diagrams clearly show hologenous conidiogenous cells with progressive proliferations (sensu Hennebert & Sutton, 1994) in figure 7 for Phyllosticta convallariae (=P cruenta (Kunze) Kickx. Although Punithalingam (1974) described P. convallariae, as the type species, he again did not give any description of conidiogenous cells. He mentioned hologenous conidiogenous cells (sensu Hennebert & Sutton, 1994) in *Phyllosticta* spp., (as Phyllostictina spp.). He was of the opinion that the two genera were not congeneric but while his paper was in press he received the type specimen of P. convallariae, and subsequently changed his mind, placing *Phyllostictina* Sydow in synonomy with *Phyllosticta*.

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Punithalingam (1974, 1981) described the spermatial stage in *Phyllosticta* without details of spermatiogenesis. Nag Raj (1983) in dealing with other *Phyllosticta* spp., showed hologenous conidiogenous cells (sensu Hennebert & Sutton, 1994). Punithalingam & Woodhams (1982) described hologenous and determinate (non-proliferating) or progressive proliferations (sensu Hennebert & Sutton, 1994) in *P. convallariae* and spermatial development, as enterogenous and stationary. Yip (1987) also illustrated hologenous and progressive conidiogenous cells (sensu Hennebert & Sutton, 1994) or without proliferations (determinate, sensu Sutton, 1980) and enterogenous and stationary spermatial development (phialidic sensu Sutton, 1980) in *P. tortilicaudata* and *P. beaumarisii*.

Punithalingam (1981, 1983, 1989) and Punithalingam & Woodhams (1982, 1984, 1986), emphasized the importance of Leifson's stain in revealing the exact nature and morphology of appendages and mucilaginous sheaths in *Phyllosticta* spp., and other fungal taxa. Punithalingam & Woodhams (1982) classified *Phyllosticta* spp., in three groups:

- 1. Appendages fairly stout and sometimes surrounded by a membranous sheath.
- 2. Appendages long and flexible, only at the base covered by a mucilaginous sheath.
- 3. Appendages short and narrow, easily breaking off from the conidium.

Punithalingam (1989) considered the appendages of *Phyllosticta* as enucleate, tubular and filiform but in some species such as *Phyllosticta theacearum* Van der Aa (IMI 31097) the appendage is much wider than normal. Abbas *et al.*, (1997) also gave and appendages classification and according to them *Phyllosticta* spp., can be classified in the following groups:

- 1. Cellular, unicellular, anucleate, blastic, holoblastic, simple, apical, hyaline, solitary;
- 2. Acellular, non-motile, mucoid, parietal, partial apical, hyaline, solitary.
- 3. Acellular, non-motile, mucoid, inclusive, hyaline, remaining soft.

In type specimen of *Phyllosticta convallariae* Persoon, appendage is apical, cellular, anucleate. So now it is a valid point as to what will be the fate of *Phyllosticata* spp., having acellular, mucoid, apical, parietal or inclusive apendage. Should they be kept in *Phyllosticata* spp., or placed in another taxa?

Bissett (1986) suggested that the correct teleomorphic generic name for *Phyllosticta* is *Discochora* Höhn., due to its priority of publication over *Guignardia* Sacc. & Sydow.

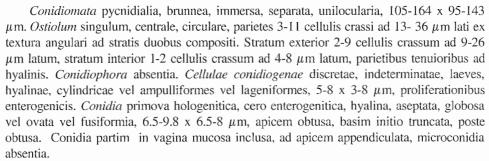
Phyllosticta Persoon

Conidiomata pycnidial to eustromatic, single to aggregated, unilocular to multilocular with a single ostiole, wall generally composed of two layers, the outer darker and thicker than the inner layer which is hyaline and thin, rarely the wall is single-layered. Conidiophores generally absent. Conidiogenous cells cylindrical to lageniform, determinate, discrete, non-proliferating or sometimes proliferate progressively or stationary. Conidia generally of two types. Macroconidia hologenous 0-1 septate, hyaline, smooth, guttulate, with or without one apical cellular or acellular, enucleate sometime mucilaginous sheath enclosing it completely or partially. Microconidia aseptate, small, without apical appendage or enclosing mucilaginous sheath function as spermatia, while spermatiogenesis, as enterogenous and stationary.

Sp. typ.: Phyllosticta convallariae (P. convallariicola) Persoon

i. Phyllosticta sultanii sp. nov.

Fig. 1



In ramis emortuis *Crotolariae burhiae*, Karachi, Pakistan, 28 Dec. 1986, S.Q. Abbas UCMH 568 (IMI 321788), holotypus.

Phyllosticta sultanii sp. nov.

Fig. 1.

Conidiomata pycnidial, brown, immersed, solitary, $105-164 \times 95-143 \mu m$, unilocular, ostiole single, circular, central, wall of textura angularis 3-11 cells thick, $13-36 \mu m$ wide, generally differentiated into two layers, an outer one thick-walled, black, 2-9 cells thick and $9-26 \mu m$ wide, and an inner one thin-walled, hyaline, 1-2 cells thick and $4-8 \mu m$ wide. Conidiophores absent. Conidiogenous cells cylindrical to ampulliform to lageniform, hyaline, smooth, $5-8 \times 3-8 \mu m$, enterogenous proliferating stationary. Conidia first formed hologenous later on enterogenous, aseptate, hyaline, globose to oval to fusoid, $6.5-9.5 \times 6.5-8 \mu m$, apex obtuse, base initially truncate but becoming obtuse, partly enclosed in mucilaginous sheath and with a small apical appendage, appendage cellular, a nucleate mucoid, paritial, apical (sensu Abbas et al., 1997).

Studies of mature conidia of P. sultanii by Nomarski interference and phase contrast microscopy showed that conidia are without a mucilaginous sheath and except in few cases there was no indication of an apical appendage. However using modified Leifson's technique (Punithalingam & Woodhams, 1982), an apical appendage as well as a mucilaginous sheath around some conidia was evident. This species fits into the 3rd group of Punithalingam & Woodhams (1982). One of the main features of this species is the presence of a vacuole which nearly fills the whole cavity of the conidium, thus distinguishing it from other species with vacuolate conidia. Besides this, P. sultanii is also characterised by the enterogenous and stationary proliferating conidiogenous cells. Punithalingam & Woodhams (1982), reported hologenous and progressive proliferating conidiogenous cells (sensu Hennebert & Sutton 1994) in P. convallariae (=P. convallariicola). Nag Raj (1983) observed hologenous and progressive proliferating conidiogenous cells in Phyllosticta sp., in the type collection of Strasseria vincae in LISE 41880. However, his diagram clearly indicates that conidiogenous cells proliferate enterogenous and progressively. Yip (1987), reported hologenous and progressive or stationary proliferating conidiogenous cells in P. tortilicaudata and P. beaumarisii.



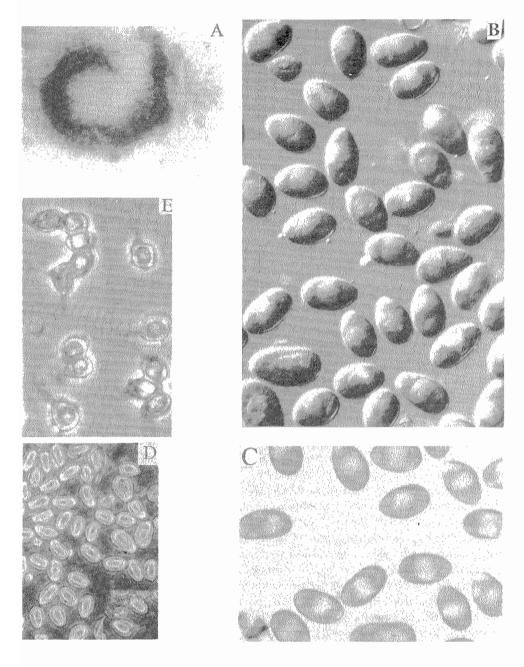


Fig. 1. *Phyllosticta sultanii* (A) V.S. of conidioma, 300X; (B) Conidia in Nomarkski optics, 1800X; (C) Conidia in bright field, 1800X; (D) Conidia stained in Leifson's flagella stain, showing apical appendage, 1800X; (E) Conidia in phase contrast, 1000X.

Phyllosticta sultanii found on Crotalaria burhia from Karachi, Pakistan was not only compared with described Phyllosticta spp., and Phoma sp., on Crotalaria and other taxa of Leguminosae but also with Phyllosticta spp., in which appendages are lacking or poorly developed.

Phyllosticta crotalariae Sacc., was described by Saccardo (1912) on Crotalaria straitae. Conidiomata are pycnidial, black, immersed, 90-130 μm, and conidia ellipsoidal, hyaline, 6-9 x 2-3 μm. Spegazzini (1922) also described Phyllosticta crotalariae Speg., from leaves of Crotalaria incana, with pycnidial conidiomata 120-160 μm and hyaline to light green conidia 3-4 x 1.5-1.75 μm. Phoma crotolariae Wehm., was described by Wehmeyer (1964) on Crotalaria albida Heyneex ex Roth. Type material was examined and gave slightly higher dimensions, conidiomata 200-400x150 200 μm, while conidia were found to be hyaline, aseptate, cylindrical, tapered at one end, 15- $18x2.5-3.0 \mu m$.

According to the rules of botanical nomenclature, *Phyllosticta crotalariae* Sacc., (1912) has priority over *Phyllosticta crotalariae* Speg., (1922). The latter is a homonym and is distinct taxon, and by its smaller conidia, clearly differs from *P. crotalariae* Sacc. Further its smaller conidial size strongly suggest that it belongs to *Phoma* sp., therefore, it is necesary to carry out cultural studies before deciding its fate.

P. sultanii has pycnidial conidiomata and resembles Phyllosticta crotalariae Sacc., Phyllosticta crotalariae Speg., Phyllosticta minima (Berk. & Curt.) Underw. & Earle, Phyllosticta minor Ell. & Everh., Phyllosticta podophylli (Curt.) Wint., and Phoma crotalariae Wehmeyer in having unilocular pycnidial conidiomata. However Phyllosticta hymenaeae (Batista & Vital) Van der Aa and P. ahmadii nom. nov., have eustromatic conidiomata. Similarly conidiomata in P. minima (125 µm), P. minor (70-120 µm), P. podophylli (80-120 μm), P. murrayicola (75-120 μm), P. crotalariae (90-130 μm) and P. hymenaeae (60-130 μm) have smaller conidiomata, while P. ahmadii (245-690 x 198-330 μm) and Phoma crotalariae Sacc., (200-400 x 150-200 μm) have larger conidiomata than P. sultanii (105-164 x 95-143 μm). The conidiomatal wall is 3-11 cells thick in P. sultanii, whereas it is 1-5 cells thick in P. moravica, P. minor, P. minima, P. podophylli, P. cyamopsidicola, P. hymenaeae and P. murrayicola, and 8-16 cells thick in P. ahmadii. Conidiogenous cells are found to be determinate or proliferate enterogenous and stationary in P. sultanii and rarely in P. ahmadii whereas in the other Phyllosticta spp., mentioned above they are non-proliferating or proliferating progressively (Punithalingam & Woodhams, 1982; Van der Aa, 1973). Conidia in P. sultanii are characterised by a large vacuole and thus resemble P. minor while conidia in all other Phyllosticta spp., have a large number of small guttules. Conidia in P. minor, P. minima, P. podophylli and P. hymenaeae are ovoid to globose to pyriform and resemble P. sultanii, whereas conidia in P. cyamopsidicola and *Phoma crotalariae* are cylindrical and can easily be distinguished from conidia of *P. sultanii*. Conidial dimensions also vary in the different *Phyllosticta* spp., mentioned above, and differ from those of *P. sultanii*. *P. moravica* (5-8 x 4.5-5.5 μm), *P. minor* (6-8 x 5-7 μm), *P.* crotolariae Speg., (3-4 x 1.5-1.75 μm) have smaller, narrower conidia, whereas conidia in P. podophylli (7-10 x 5-7 μm), P. minima (7.5-12 x 4.5-7.4 μm), P. cyamopsidicola (8-12 x 5-7.5 μm), P. hymenaeae (6-10 x 4-7 μm) and Phoma crotalariae (15-18x2.5-3.5 μm), have slightly larger narrower conidia. In P. moravica (7-13 x 5-10 μm) and P. ahmadii (18-5-26.5 x 18.5-21.5 \(\mu\)m), conidia are longer and wider than in P. sultanii (6.5-9.5 x 6.5-8 \(\mu\)m). Though the apical appendage is characteristic of the genus *Phyllosticta*, many species lack an



appendage or the appendages are poorly developed. *P. sultanii* comes in category III of Punithalingam & Woodhams (1982). Under bright field and Nomarski interference microscopy the appendage is not visible in *P. sultanii* but it is visible under phase contrast when it becomes more evident by using modified Leifson's flagella staining technique (Punithalingam & Woodhams, 1984). It is important to note that a small quantity of conidia do have an apical appendage, so *P. sultanii* can easily be distinguished from *P. hymenaeae*, *P. cyamopsidicola* and *P. podophylli*, where the majority of conidia have a prominent apical appendage. It resembles *P. moravica*, *P. minima* and *P. minor* where no apical appendage has been found in the herbarium material examined. In cultures however, *P. minima* and *P. moravica* do develop an apical appendage. In *P. murrayicola* instead of a prominent apical appendage, there is a mucilaginous residue present at the apex. In the same way, *P. ahmadii*, can easily be distinguished from *P. sultanii* in having a prominent well-developed mucilaginous sheath around conidia and in the absence of an apical appendage.

Specimen examined

Phyllosticta sultanii sp. nov.

On twigs of *Crotalaria burhia*, Karachi, Pakistan, 28 Dec., 1986, S.Q. Abbas UCMH 568 (IMI 321788), holotype.

ii. Phyllosticta ahmadii nom. nov.Fig. 2.

Specimens of Capparis decidua collected by S.Q. Abbas from Karachi and those of Sultan Ahmad from the Punjab yielded two fungi, one with eustromatic conidiomata, aseptate, hyaline, oblong conidia enclosed in a thick mucilaginous sheath, and the other also with eustromatic conidiomata but muriform conidia enclosed in mucilaginous sheaths. The former has been described as Botryodiplodia ambigua Petrak, the latter as Camarosporium capparidis Ahmad. Petrak & Ahmad (1954) described Botryodiplodia ambigua with a doubt as to whether it belonged to Phyllostictina (=Phyllosticta) or Botryodiplodia. Detailed studies of the type specimen of B. ambigua showed that it belonged to Phyllosticta. In the present study this fungus has been renamed as P. ahmadii in honour of Prof. Sultan Ahmad since name Phyllosticta ambigua is already preoccupied. Further studies on Camarosporium capparidis Ahmad, (Abbas et al., 2000) showed that conidia had mucilagenous sheath around them which was absent in conidia of Camarosporium therefore they erect a new genus Camarosporiopsis.

Phyllosticta ahmadii nom. nov. Fig. 2.

Botryodiplodia ambigua Petrak, Sydowia 8: 173 (1954), non Phyllosticta ambigua Scalia, Sul seccume del Frassino de Manna, Catania: 13, (1909), nec Phyllosticta ambigua Sacc., Annls. mycol., 10: 317 (1912).

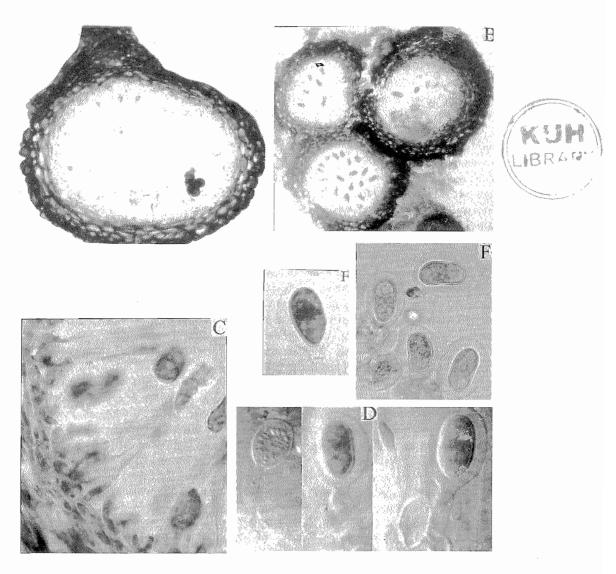


Fig. 2. Phyllosticta ahmadii, IMI 3188494 (A) V.S. of simple conidioma, 100X; IMI 318510 (B) V.S. of aggregated conidiomata, 100X; IMI 138587 (C) Conidiomatal wall, conidiogenous cells and paraphyses, 1000X; (D) Conidiogenous cells, 1000X; IMI 318497 (E) Conidia enclosed in mucilaginous sheath, 1000X; IMI 138510 (F) Conidium with prominent mucilaginous sheath.

Conidiomata eustromatic, black, initially immersed, later becoming erumpent, ostiolate, unilocular to multilocular, 245-690 x 198-330 μ m. Wall 8-16 cells thick of textura angularis, 32-80 μ m wide, generally differentiated into two layers, the outer thicker and darker than the inner one which is thin and hyaline. Conidiophores mostly absent, but when present then 3 septate, cylindrical, hyaline and smooth. Conidiogenous cells cylindrical to lageniform, hyaline, smooth, determinate sometimes proliferating enterogenous and stationary 6.5-12x2.5

 μ m. Conidia formed first hologenous, later on enterogenous hyaline, obtuse at apex and base, slightly constricted in the middle, with a prominent mucilaginous sheath around the conidia, 13-22.5x7-11 μ m without sheath, and 18.5-26.5x18.5-21.5 μ m with sheath. The mucilaginous sheath not only encloses the conidia and conidiogenous cells but also the conidiophores when present. Microconidia was not present.

Phyllosticta species reported from Capparis species are P. capparidis Saccardo & Spegazzini (Saccardo, 1878), P. capparidicola Spegazzini (1910), P. capparidearum Spegazzini (1910), P. densissima Saccardo (1916), P. delhiensis Chona & Munjal (1956), P. capparidis-hyneanae Da Costa & Mundkur (1948) and P. capparidis Chardon (1938) (without Latin diagnosis). All these Phyllosticta spp., clearly differ from P. ahmadii. Conidiomata in P. ahmadii are eustromatic, unilocular to multilocular, 245-690 x 198-330 μ m whereas the conidiomata of the other species are generally pycnidial and not more than 300 μ m. Similarly the conidia of P. ahmadii are comparatively large 18.5-26.5x18.5-21.5 μ m with a mucilaginous sheath compared with the other species in which there is no description of any mucilaginous sheath or apical appendage. In addition P. ahmadii has paraphyses which are absent in the rest. Indeed, the small conidial sizes of the described Phyllosticta species on Cappris spp., indicate that they should more correctly be referred to Phoma Sacc.

Saccardo (1878) published *Phyllosticta capparidis* Saccardo & Spegazzini. Later, Chardon (1938) also published *P. capparidis*, without a Latin diagnosis, therefore it is an invalid name, though conidiomatal and conidial size of both taxa are significantly different. Before proposing a new name, studies of the type specimens is necessary.

Phyllosticta ahmadii has also some similarities with other Phyllosticta spp., in the conidial size. In P. ahmadii conidia measure 18.5-26.5 x 18.5-21.5 µm compared with P. sphaeropsoidea Ell. & Everh., (10-20 X 9-13 µm), P. concinna (Syd.) Van der Aa (12-20 x 7-10 μm), P. musarum (Cooke) Van der Aa, (10-20 X 7-13 μm), P. cruenta (Kunze: Fr.) Kickx (12-21 X 5-10 μm). Conidia of P. concentrica Sacc., (8-20 x 4-14 μm) resemble P. ahmadii more closely, however, these Phyllosticta spp., differ from P. ahmadii in having an apical appendage with mucilaginous sheath and greenish guttules in the conidia. P. ahmadii also resembles P. hamamelidis Cooke, P. pyrolae Ell. & Everh., P. colocasiicola Höhn., P. arxii Van der Aa, in the absence of an apical appendage. However, there are reasonable differences which separate them. P. arxii is leaf-inhabiting with smaller conidiomata (80-300 μ m), narrow conidiogenous cells (4-12 x 1.5-2.5 μ m) and slightly smaller and thinner conidia (6-18 x 2-8 µm). Similarly P. colocasiicola also has small conidiomata (100-120 μ m), however in culture it produces larger eustromatic conidiomata (800 μ m). Further it has very short conidiogenous cells and small conidia (9-13 x 4-6 µm), P. pyrolae is also a leaf-inhabiting fungus with smaller conidiomata (80-180 µm), short and narrow conidiogenous cells (5-9 x 2-3 μ m) and very small conidia (5-9 x 2-3 μ m).

Phyllosticta flavolandii also resembles *P. ahmadii* in having large conidiomata (up to 750 μ m), and walls 15-35 μ m thick, conidiogenous cells (8-12 x 2-35 μ m), and branched conidiophores, however it clearly differs by its smaller conidia (11-14 x 7-9 μ m) than *P. ahmadii* (18.5-26 x 18.5-21.5 μ m).

There are also three *Phoma* species reported from *Capparis* spp. In *P. herbarim* forma capparidis Saccardo (1884) conidia are cylindrical, hyaline, 7-9 x 4 μ m, and in *P. capparidis* Passerini, conidiophores are 20-25 μ m and conidia are 1.0-2.5 μ m. In *P. capparidina* Passerini conidia are 1.25 x 5 μ m (Saccardo, 1892). All these *Phoma* spp., can easily be distinguished from *P. ahmadii* in having small conidia without sheaths.

Phyllosticta ahmadii has similarities with Botryodiplodia Sacc., but is easily distinguished by the aseptate conidia and presence of mucilaginous sheath around the conidia. P. ahmadii can also be separated from Avettaea Petrak & Sydow in having hyaline rather than brown conidia.

Specimens examined

Phyllosticta ahmadii nom. nov.

On twigs of *Capparis decidua* (as *C. aphylla*, Sheikupura, Pakistan 18 Sept. 1950, S. Ahmad Ex, F. Petrak, Pilzherbarium, (IMI 318497), holotype (= holotype of *Botryodiplodia ambigua* Petrak), Changa manga, Pakistan, 24 Feb. 1962, S. Ahmad 15468 (IMI 93028); Jehlum, Pakistan, 21 July 1964, S. Ahmad 18131, (IMI 138510); Karachi, Pakistan, 28 Dec., 1985, S.Q. Abbas UCMH No. 288; Kharian, Pakistan, 17 July 1964, S. Ahmad 181171 (IMI 13336); Lahore, Pakistan, 5 Dec. 1975, S. Ahmad 15053 (IMI 199880).

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