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Full Length Article

Species Diversity of Genus *Puccinia* (Basidiomycota, Uredinales) Parasitizing Poaceous Hosts in Pakistan

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Abstract

To explore the distribution of graminicolous rust fungi in Pakistan, the Azad Jammu and Kashmir, Khyber Pakhtoonkhaw and Northern Areas were surveyed. Seven *Puccinia* species were found parasitizing nine host plants belonging to Poaceae. Among these, the telial stage of *P. brachypodii* var. *arrhenatheri* is a new record for Pakistan. In addition, the following are new poaceous hosts for rust fungi in Pakistan: *P. agrostidis-caninae* on *Agrostis gigantea and A. stolonifera, P. brachypodii* var. *arrhenatheri* on *Koeleria macrantha, P. brachypodii* var. *brachypodii* on *Piptatherum laterale, P. brachypodii* var. *poae-nemoralis* on *A. gigantea and Poa polycolea, P. coronata* var. *himalensis* on *Helictotrichon virescence* and *Sporobolus arabicus, P. graminis* subsp. *graminicola* on *Agropyron striatum* and *P. versicolor* on *Themeda anathera*. In addition to light microscopy, Scanning Electron Microscopy (SEM) was utilized to examine wall ornamentation of spores for accurate identification.

Keywords: Pakistan; Poaceous hosts; *Puccinia* spp.; Rust fungi; Scanning electron microscopy

Introduction

Puccinia is the largest genus of Uredinales, with about 3000 to 4000 species. The Asteraceae, Cyperaceae, Liliaceae and Poaceae serve as hosts for many species, but most groups of vascular plants are parasitized (Cummins and Hiratsuka, 2003). Many serious diseases are caused by species of *Puccinia*. These include; black stem rust of wheat (*P. graminis* Pers. ex Pers.), common corn rust (*P. sorghi* Schwein) and sugarcane rust (*P. melanocephala* Syd.).

In Pakistan, about 158 genera and 492 species of *Poaceae* are known of which 130 species are reported as hosts for more than 100 species of rust fungi, including 70 species of *Puccinia* and 14 species of *Uromyces* (Afshan and Khalid, 2009; Afshan *et al*., 2009; Iqbal *et al*., 2009; Khalid and Afshan, 2009; Afshan *et al*., 2010ab, 2011; Hussain *et al.*, 2011). Poaceous weeds are important competitors of crop plants worldwide as well as in Pakistan. The weeds that invade economically important grass crops cause significant harvest loss. Host specific rust fungi have been used as biological control agents against noxious weeds (Yandoc-Ables *et al*., 2006).

Materials and Methods

During the survey of rust fungi from Pakistan, infected plants were collected from different areas of Pakistan including Khyber Pakhtoonkhaw (Jalkhud, Khanspur, Mukshpuri and Sharan), Azad Jammu and Kashmir (Lawat and Sharda) and Northern Areas (Fairy Meadows). Healthy plants were collected along with inflorescences and fruits for accurate identification. Host plants were identified by comparing them with specimens in the herbarium of the Department of Botany, University of the Punjab, Lahore (LAH), Pakistan.

Free hand sections of infected portions of material and spores were mounted in lactophenol. Semi-permanent slides were prepared by cementing cover slips with nail lacquer (Dade and Gunnell, 1969). Preparations were observed under a NIKON YS 100 microscope. Drawings of spores were made by using a Camera Lucida (Ernst Leitz, Wetzlar, Germany). Spores were measured using an ocular micrometer (Zeiss, St Albans, Hertfordshire, England). At least 25 spores were measured for each spore stage. Measurements include the usual range and the arithmetic means; extremes are given in parentheses.

Results and Discussion

P. agrostidis-caninae

Afshan, Berndt, Khalid and Niazi, *Mycotaxon* 104: 123, (2008) (Fig. 1).

Spermogonia and aecia not seen. Uredinia amphigenous, subepidermal, golden brown to blackish brown, present in rows, scattered but somewhat compact, naked, 0.06–0.11 × 0.094–2 mm. Urediniospores globose to subglobose, ellipsoid or obovoid, hyaline to light brown,

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(15–) 17–19 (–20) × 17–22 (–24) µm; wall 1.5–2 (–3) µm, echinulate, hyaline; germ pores obscure, 4–9, scattered; paraphyses absent. Telia on adaxial side of leaf, compact, present in rows, blackish, naked, tending to be loculate, surrounding by stromatic paraphyses, 0.04–0.08 × 0.06–0.14 mm. Teliospores 1–2 celled, clavate to ellipsoid, chestnut brown at the apex and cinnamon brown to paler basally, slightly constricted at the septum, two-celled teliospores (15–) 16–20 (–22) × (27–) 30–54 (–60) µm; wall 1–1.5 µm thick, smooth; one-celled teliospores clavate or ellipsoid, 13–25 × 27–31 µm; apex conical, obliquely conical or truncated, sometimes rounded, chestnut brown, 3–4 µm thick; pedicel short, persistent, light brown to dark brown, 6–9 × 4–10 µm; paraphyses clavate, brown, 3.3–6 × 31–51 µm.

Material examined: On *Agrostis gigantea* Roth, with II + III stages, Pakistan, Khyber Pakhtoonkhaw (KPK), Mukshpuri track, at 2813 m a.s.l., 16th September, 2006. Malka Saba # 18 (LAH); On *A. stolonifera* L., Pakistan, Khyber Pakhtoonkhaw (KPK), Khanspur, at 2135 m a.s.l., 10th July, 2008. Malka Saba # 19 (LAH); On *A. stolonifera* L., Pakistan, Khyber Pakhtoonkhaw (KPK), Sharan, at 2752 m a.s.l., 27th July, 2007. Malka Saba # 20 (LAH).

Comments: *P. agrostidis-caninae* has previously been reported on *A. canina* L. from Khanspur-village by Afshan *et al.* (2008a). *A. gigantea* and *A. stolonifera* are new hosts for this rust fungus in Pakistan.

P. brachypodii* var. *arrhenatheri

Cummins and H.C. Greene, *Mycologia* 58: 709 (1966) (Figs. 2–3).

Spermogonia and aecia not seen. Uredinia on abaxial side of leaf, golden brown, scattered, solitary, subepidermal, covered by ruptured epidermis. Urediniospores globose, hyaline to light brown, 17–24 × 20–27 µm; wall 1–1.5 µm thick, echinulate; germ pores upto 8, obscure, scattered; paraphyses capitate, abundant, constricted at the neck, hyaline to light brown, 30–70 µm long, apex 10–16 µm wide while 5–10 µm wide at the base, wall 1–1.5 µm thick. Telia amphigenous, black, scattered, subepidermal. Teliospores cinnamon brown to light brown at the base but chestnut brown at the apex, constricted at the septum, 14–26 × 27–43 µm; wall 1–1.5 µm thick, smooth; apex chestnut brown, truncated or obliquely conical, sometimes rounded, 3–4.5 (–6.5) µm thick; pedicel short, persistent, light brown to dark brown, sometimes obliquely attached, 5–7.5 × 11 µm.

Material examined: On *Koeleria macrantha* (Ledeb.) Schult., with II + III stages, Pakistan, Northern Areas, Fairy Meadows, at 3,036 m a.s.l., 23 July, 2010. Malka Saba # 21 (LAH).

Comments: Telial stage of *P. brachypodii* var. *arrhenatheri* is a new record for Pakistan. The uredinial stage of this rust has previously been reported from Khanspur by Afshan *et al*. (2008b).

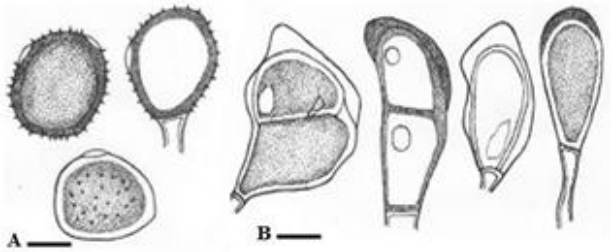

Fig. 1: Lucida drawings of *P. agrostidis-caninae* **(A)** Urediniospores showing germ pores and echinulate ornamentation **(B)** Teliospores. Scale bar: A = 8 µm and B = 10 µm

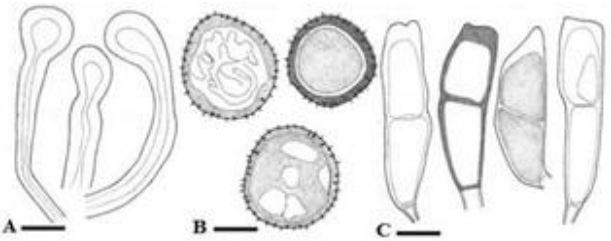

Fig. 2: Lucida drawings of *P. brachypodii* var. *arrhenatheri* **(A)** *Capitate paraphyses* **(B)** Urediniospores showing echinulate ornamentation **(C)** Teliospores. Scale bar = 10 µm

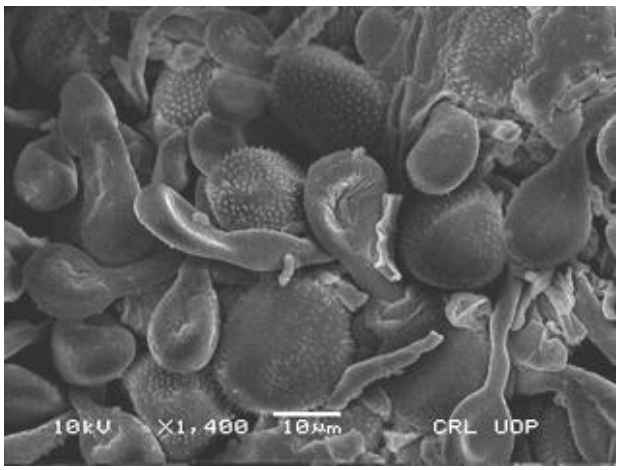

Fig. 3: Uredinium of *P. brachypodii* var. *arrhenatheri* as seen by Scanning Electron Microscopy showing urediniospores and *Capitate paraphyses*.

P. brachypodii* var. *brachypodii

G.H. Otth, *Mitt. naturf. Ges. Bern*: 81 (1861) (Figs. 4–5).

Spermogonia and aecia not seen. Uredinia amphigenous, scattered, cinnamon brown, covered by ruptured epidermis, naked, 0.07–0.1 × 0.09–0.2 mm. Urediniospores globose to sub-globose or ellipsoid, pale brown with yellowish contents, (18–) 20–27 × (21–) 24–29 (–32.5) µm (mean 22.8 × 26.4 µm), wall 1.5–2.5 µm,

echinulate; germ pores 7–10 (–11), scattered, sometimes appeared as tending to be equatorial, lumen often invigilated at the pores; pedicel hyaline, deciduous; *Paraphyses capitate*, abundant, 34–68 µm long, 9–21 µm wide apically, 4–10 µm at the base, thickened apically, rarely cylindrical. Telia on abaxial side, blackish, scattered, sub-epidermal, tending to be loculate with brown peripheral paraphyses. Teliospores oblong or ellipsoid, cinnamon brown, fragile, easily broken, slightly constricted at the septum, attenuated below, (18–) 20–24 (–26) × (27–) 30–38 (–40) µm (mean 21.8 × 32.2 µm); wall smooth, 1–1.5 (–2) µm; apex dark brown to chestnut brown, truncated or conical, 2–5 µm thick; pedicel persistent, dark brown, short 8–10 × 7 µm. Mesospores rarely present.

Material examined: On *Piptatherum laterale* (Munro ex Regel) Rozhev. (= *Milium laterale* Munro ex Regel and *Oryzopsis pubiflora* Hack.), with II + III stages, Pakistan, Azad Jammu and Kashmir, Neelum Valley, Sharda, at 1,981 m a. s. l., 16 August, 2009. Malka Saba # 22.

Additional material examined: On *Brachypodium sylvaticum* P. Beauv., with II + III stages, Germany, Bayreuth, Laubwald bei der Eremitage, 6 October, 1918, leg. H. Poeverlein, det. N. H. Paul, ZT (ZT Myc 1143).

Comments: *P. laterale* is a new host for *P. brachypodii* var. *brachypodii*. This rust fungus has been reported on *Brachypodium flexum* Nees, *B. pinnatum* (L.) P. Beauv., *Brachypodium* sp., *B. sylvaticum* P. Beauv., *B. sylvatium* P. Beauv. var. *braviglume* Keng ex Keng f. and *Trachynia distachya* Link. (Farr and Rossman, 2011).

P. brachypodii* var. *poae-nemoralis

(G.H. Otth) Cummins and H.C. Greene, *Mycologia* 58(5): 705 (1966) (Fig. 6).

Spermogonia, aecia and telia not seen. Uredinia amphigenous, dark brown. Urediniospores globose to ellipsoid, hyaline to light brown, 16–23 × 18–26 µm; wall 1–2 µm thick, echinulate; germ pores 8–12, obscure, scattered. Urediniospores intermixed with paraphyses, paraphyses cylindrical to capitate, 40–82 µm long, 8–17 µm wide at the upper end, 4–7 µm wide at the lower end, wall 2–4 µm throughout or to 7 µm thick in the head. Telia on abaxial side, blackish, scattered, covered by epidermis. Teliospores oblong or clavate, light brown to pale brown at the base and chestnut brown at the apex, 17–31 × 34–57 µm, wall smooth, 1.4–2.4 µm, constricted at the septum, attenuated at the base, one germ pore in each cell, apex chestnut brown, truncated or conical, rarely obliquely conical, 2–7 µm; pedicel short, pale to dark brown, persistent, 5–9 × 5–10 µm.

Material examined: On *Poa polycolea* Stapf., with II + III stages, Pakistan, Khyber Pakhtoonkhaw (KPK), Jalkhud, at 3,300 m a. s. l., 16th August, 2009, Malka Saba # 23.

Comments: *P. brachypodii* var. *poae-nemoralis* has previously been reported on *A. munroana* from Batakundi (Kaghan valley) by Ahmad (1956a) and on *P. nemoralis* L., *P. pratense* L., *P. sterilis* from Sharan, NWFP, Swat state and AJ and K by Kakishima *et al.* (1993a, b) and Masood *et al*. (1995).

P. brachypodii var. *poae-nemoralis* is a new record from Jalkhud and *P. polycolea* is a new host for this rust fungus.

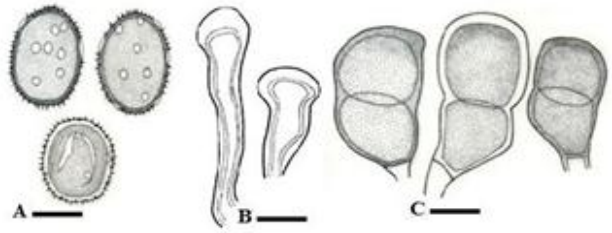

Fig. 4: Lucida drawings of *P. brachypodii* var. *brachypodii* **(A)** Urediniospores showing echinulate ornamentation and germ pores **(B)** Capitate paraphyses **(C)** Teliospores. Scale bar = 10 µm

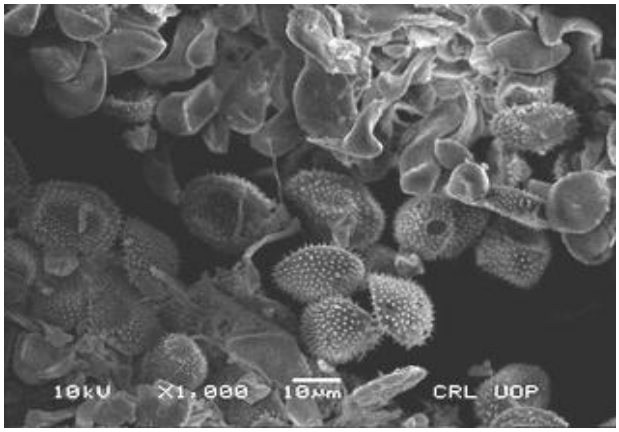

Fig. 5: Uredinium of *P. brachypodii* var. *brachypodii* as seen by Scanning Electron Microscopy showing urediniospores with echinulate ornamentation and *Capitate paraphyses*

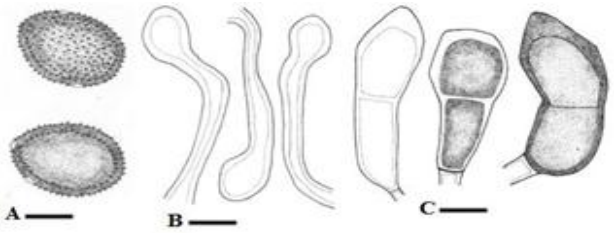

Fig. 6: Lucida drawings of *P. brachypodii var. poae-nemoralis* **(A)** Urediniospores showing echinulate ornamentation and scattered position of germ pores **(B)** *Capitate paraphyses* **(C)** Teliospores. Scale bar: 10 µm

***P. coronata* var. *himalensis*:**

Barclay *Trans. Linn. Soc. London* 3: 227 (1891) (Fig. 7).

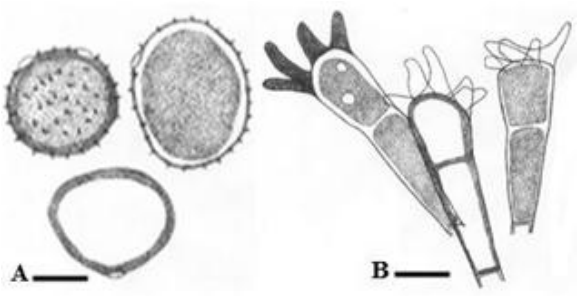

Fig. 7: Lucida drawings of *P. coronata* var *himalensis* **(A)** Urediniospores showing echinulate ornamentation **(B)** Teliospores. Scale bar: A = 8 µm, B = 10 µm

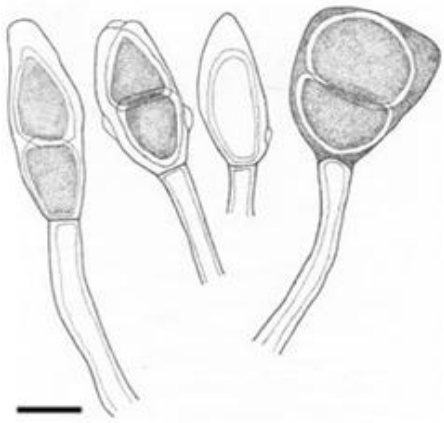

Fig. 8: Lucida drawing of *P. graminis* subsp. *graminicola* showing 1–2 celled teliospores. Scale bar: 10 µm

Spermogonia and aecia not seen. Uredinia amphigenous, brown, scattered but somewhat compact. Urediniospores globoid to ovoid, hyaline to light brown, 14–20 × (16–) 18–22 µm; wall upto 1 µm thick, hyaline, echinulate; germ pores obscure, 2–5, scattered or tending to be equatorial; paraphyses scanty, clavate to cylindrical, hyaline, mostly at the periphery. Telia amphigenous, black, without paraphyses. Teliospores 1–2 celled, ellipsoid to cylindrical, cinnamon brown to pale brown at the base, while chestnut brown at the apex, 14–18 × 24–39 µm excluding digitations; wall 1–1.5 µm thick, smooth; digitations 2–13 µm long; pedicel short, light brown, persistent, 8 µm long.

Material examined: On *Sporobolus arabicus* Boiss., with II + III stages, Pakistan, Khyber Pakhtoonkhaw (KPK), Sharan, at 2752 m a.s.l., 27th July 2007. Malka Saba # 24 (LAH); On *Helictotrichon virescens* (Nees ex Steud.) Henrard, with II + III stages, Pakistan, Khyber Pakhtoonkhaw (KPK), Sharan, at 2752 m a.s.l., 27th August, 2008. Malka Saba # 25 (LAH).

Comments: *P. coronata* var. *himalensis* is a new record for Pakistan. *S. arabicus* and *H. virescens* are also new hosts for this rust fungus from Pakistan.

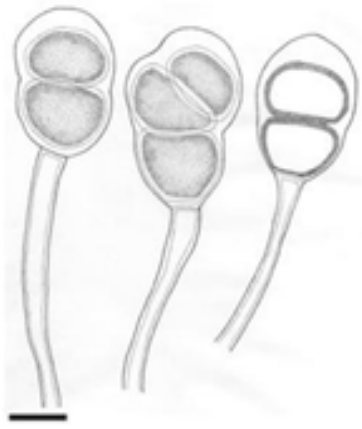

Fig. 9: Lucida drawings of *P. versicolor* showing 1–2 celled teliospores. Scale bar = 15 µm

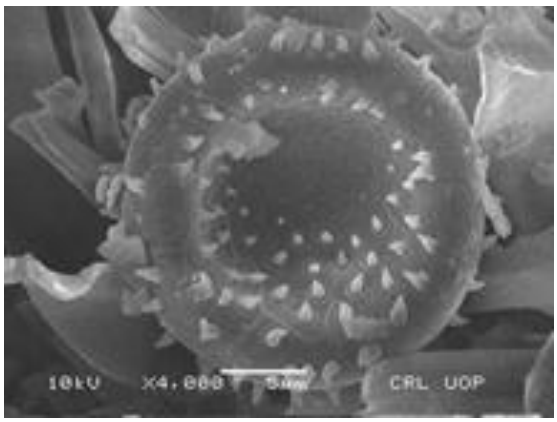

Fig. 10: Urediniospore of *P. versicolor* as seen by Scanning Electron Microscopy showing echinulate ornamentation

P. graminis*: subsp. *Graminicola

Z. Urb., *Ceska Mykol.* 21: 14 (1967) (Fig. 8).

Spermogonia, aecia and uredinia unknown. Telia on sheaths and stems, early exposed, blackish brown, compact. Teliospores 1-2 celled, oblong to ellipsoid, chestnut brown at the apex and cinnamon brown to dark brown at the base, constricted at the septum, two celled teliospores 14–21 × 26–56 µm; one celled teliospores 11–19 × 24–47 µm; wall 1.5–2 µm thick, smooth; apex chestnut brown, conical, truncate, sometimes obliquely conical, 3–9 µm thick; pedicel hyaline to brownish, persistent, usually collapsing, 5–8 × 11–74 µm.

Material examined: On *Agropyron striatum* Nees ex Steud., with III stage, Pakistan, Khyber Pakhtoonkhaw (KPK), Sharan, at 2752 m a.s.l., 13th August, 2007. Malka Saba # 26 (LAH).

Comments: *P. graminis* subsp. *graminicola* has previously

been reported on *A. semicostatum* Nees., *A. munroana* Aitch. and Hemsl., *Bromus japonicus*, *Cynodon dactylon* Pers., *Hordeum vulgare* L. and *Triticum aestivum* L. by Ahmad (1956b). *P. graminis* subsp. *graminicola* is a new record from Sharan and *A. striatum* is a new host for this rust fungus from Pakistan.

P. versicolor

Dietel and Holw in Holway, *Bot. Gaz.* 24: 28 (1897) (Figs. 9–10).

Spermogonia and aecia were not seen. Uredinia similar, abaxial, black, exposed, 0.1–0.2 × 0.2–0.4 mm. Urediniospores globose to sub-globose or ellipsoid, cinnamon brown, lumen tending to be stellate, 21–27 × 25–33 µm (mean 24.2 × 28.8 µm); wall minutely and finely echinulate, 2.3–3.1 µm; germ pores up to 5, tending to be equatorial, obscure. Telia similar, abaxial, pulvinate, black, naked, exposed, 0.1–0.2 × 0.2–0.4 mm. Teliospores 1–3 celled, abundantly 2-celled, third septum may be transverse, oblique or vertical, cinnamon brown to dark brown, ellipsoid, rounded at both ends, constricted at the septum, 24–33.5 × (31.4–) 37–52.4 µm (mean 28.97 × 44 µm); wall dark brown, smooth, 1.2–1.8 (–4.5) µm; germ pores one per cell, obscure; apex rounded, dark brown to chestnut brown, (2.5–) 3–5.4 (–7.8) µm; pedicel persistent, long, hyaline to light brown, 3.6–10.6 × (10–) 18–91 (–118) µm. Three celled teliospores occurred commonly.

Material examined: On *Themeda anathera* Hack., with II + III stages, Pakistan, Azad Jammu and Kashmir, Neelum valley, Lawat, 16th October, 2009. Malka Saba # 27 (LAH).

Comments: *P. versicolor* has been reported on *Heteropogon contortus* (Linn.) Beauv. ex Roem. and Schult. from Choa Saidan Shah by Ahmad (1956a, b, 1976). Presence of 1–3 celled teliospores is contribution to the description of *P. versicolor* and *T. anathera* is a new host for this rust fungus in Pakistan.

Acknowledgments

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