

Short paper

## *Puccinia taeniatheri* a new graminicolous rust species from Iran

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**Abstract:** *Puccinia taeniatheri* sp. nov., is newly described from Iran on *Taeniatherum asperum* (Poaceae) based on morphological study and rDNA ITS sequencing. An identification key for *Puccinia* species known on the genus *Taeniatherum* is provided. Re-examination of herbarium material of *P. hordei* on *T. crinitum* collected from Iraq revealed that specimen also belongs to *P. taeniatheri* sp. nov.

**Keywords:** Pucciniales, Poaceae, Taxonomy, Biodiversity, New species

### Introduction

The knowledge of graminicolous rust fungi in Iran has risen in recent years. Abbasi (2002), Abbasi *et al.* (2002a, 2002b, 2002c, 2004, 2005) and Abbasi and Hedjaroude (2004) have made a tremendous addition to the taxonomy of rust fungi on Poaceae in Iran. So far, 39 *Puccinia* species have been reported on Poaceous plants in Iran (Abbasi, unpublished data). Here, another *Puccinia* species on a member of Poaceae family is described as a new taxon to the sciencebased on morphological and molecular data.

### Materials and Methods

#### Source of specimens

The materials for morphological studies and DNA extraction were collected by the author during the last 30 years (Table 1) and were preserved at the herbarium of the Ministry of Agriculture (Iran).

### Morphological studies

Morphological studies and microscopy were carried out using the methods described in Abbasi (2002).

### DNA extraction, PCR amplification, and sequencing

DNA was extracted according to the method of Abbasi (2002) and Abbasi *et al.* (2004). The ITS region (ITS-1, 5.8S gene, ITS-2) of each specimen was amplified with primers ITS4 and ITS5 of White *et al.* (1990). Amplification was as described by Zambino and Szabó (1993) with the following cycling parameters: 40 cycles of 94 C for 30 sec, 50 C for 1 min, 72 C for 2 min, and a final extension of 10 min at 72 C. The size and quantity of amplification products were verified on 1% agarose gels and extracted with the GeneClean spin kit (BIO 101, Vista, CA) according to the manufacturer's instructions. Purified products were cloned with TA cloning kit (Invitrogen Corp., Carlsbad, CA). Several clones per rust specimen were obtained, and the presence of inserts was confirmed by digestion with Eco RI and agarose electrophoresis. Plasmid DNA was prepared with the Wizard Miniprep Kit (Promega, Madison, WI) and DNA concentration was estimated with a fluorometer. DNA samples were sequenced with the ThermoSequenase fluorescent labeled primer

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cycle sequencing kit (Amersham Pharmacia Biotech) and sequenced on an ALFexpress automated DNA sequencer (Amersham Pharmacia Biotech) as described by Goodwin & Zismann (2001). Several clones were sequenced per rust specimen to minimize errors caused by PCR amplification.

**DNA sequence alignment and analysis**

DNA sequences were aligned with the profile mode of Clustal X (Thompson *et al.* 1997) and were edited manually when necessary. Phylogenetic tree was constructed using the neighbor-joining method with the Kimura 2-

parameter substitution model in MEGA 6.0. Branch robustness was assessed via bootstrap analysis with 1,000 replicates.

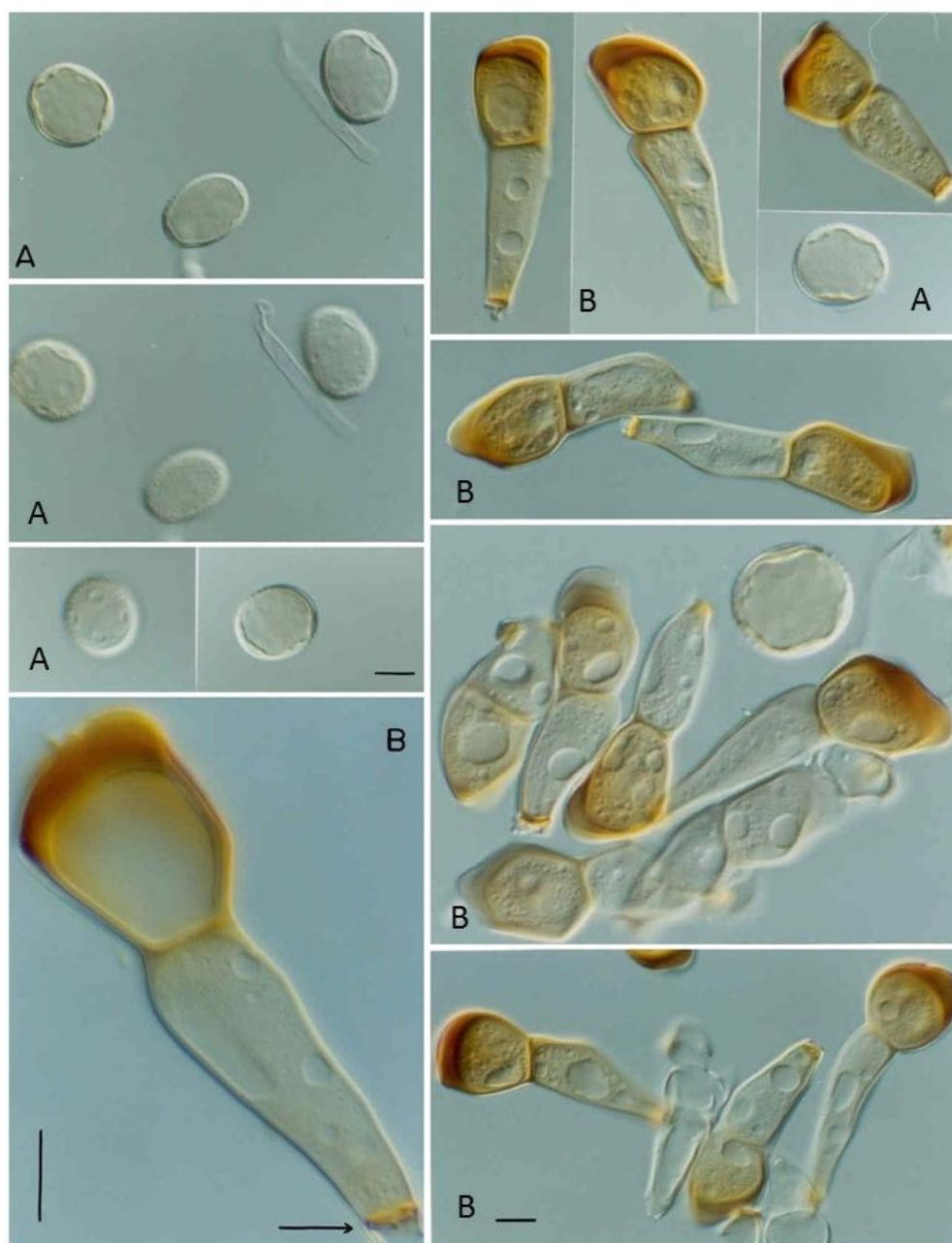
**Results and Discussion**

*Puccinia taeniatheri* M. Abbasi & Hedjar., **sp. nov.**, MB 835520 – Fig. 1

Holotype: On *Taeniatherum asperum* (Simonkai) Nevski, IRAN, Golestan prov., Golestan national park, Dasht towards Ghezghaleh, alt. 1050-1150 m, 11 May 1992, leg. M. Abbasi (IRAN 11491 F) (GenBank: ITS = AY956557).

**Table 1** Summary information for rust specimens included in the sequence analysis.

Rust species	Host species	Locality	Length of ITS region (bp)	GenBank accession nos.	Herbarium (IRAN) accession nos.
<i>Puccinia persistens</i> ssp. <i>triticina</i>	<i>Aegilops taushii</i>	Iran, Golestan prov.	697	AY956549	8274F
<i>Puccinia persistens</i> ssp. <i>triticina</i>	<i>Triticum aestivum</i>	Iran, West Azarbaijan prov., Khoy	696	AY880845	8226F
<i>Puccinia persistens</i> ssp. <i>agropyrina</i>	<i>Elymus hispidus</i> var. <i>villosus</i>	Iran, Ardabil prov., Sareyn	700	AY956552	11513F
<i>Puccinia persistens</i> ssp. <i>agropyrina</i>	<i>Thalictrum minus</i>	Iran, Alborz prov., Karaj	706	AY956561	11511F
<i>Aecidium</i> sp.	<i>Thalictrum sultanabadense</i>	Iran, Hamedan prov. Malayer	685	AY956563	11487F
<i>Puccinia bromina</i>	<i>Bromus sterilis</i>	Iran, Alborz prov., Karaj	693	AY956550	11507F
<i>Puccinia bromina</i>	<i>Bromus tectorum</i>	Iran, Golestan prov.	690	AY956548	9059F
<i>Puccinia hordei</i>	<i>Hordeum vulgare</i>	Iran, Khuzestan prov.	695	AY874150	10835F
<i>Puccinia taeniatheri</i> sp. nov.	<i>Taeniatherum asperum</i>	Iran, Golestan prov.	689	AY956557	11491F
<i>Puccinia schismi</i>	<i>Lophochloa phleoides</i>	Iran, Bushehr prov.	689	AY956555	10196F
<i>Puccinia schismi</i> var. <i>loliina</i>	<i>Lolium temulentum</i>	Iran, Golestan prov.	688	AY956554	3922F
<i>Puccinia triseti</i>	<i>Trisetum flavescens</i>	Iran, Golestan prov.	688	AY956556	11490F
<i>Puccinia recondita</i> s.str.	<i>Elymus</i> sp.	Iran, Golestan prov.	691	AY956553	11506F
<i>Puccinia recondita</i> s.str.	<i>Secale segetale</i>	Iran, Golestan prov.	691	AY956551	11505F
<i>Puccinia recondita</i> s.str.	<i>Cerintho minor</i>	Iran, Qazvin prov.	692	AY956562	11486F
<i>Puccinia striiformoides</i>	<i>Dactylis glomerata</i>	Iran, Tehran prov., Tehran	684	AY956558	9052F
<i>Puccinia striiformis</i>	<i>Triticum aestivum</i>	Iran, Alborz prov., Karaj	682	AY874152	11502F
<i>Puccinia striiformis</i>	<i>Hordeum geniculatum</i>	Iran, Kohgiluyeh and Boyer-Ahmad prov., Yasuj	683	AY956559	11497F
<i>Puccinia pseudostriformis</i>	<i>Poa pratensis</i>	Iran, Mazandaran prov.	683	AY956560	11500F
<i>Puccinia coronata</i> var. <i>avenae</i>	<i>Avena ludoviciana</i>	Iran, Mazandaran prov.	700	AY956564	11475F
<i>Puccinia trebouxii</i>	<i>Melica jacquemontii</i>	Iran, Alborz prov., Azadbar	686	AY956565	11482F
<i>Puccinia wolgensis</i>	<i>Stipa hohenackeriana</i>	Iran, Alborz prov., Dizin	686	AY956566	11481F



**Figure 1** *Puccinia taeniatheri* sp. nov., from holotype, A. urediniospores, B. teliospores, arrow shows brown septum at the point of attachment of pedicel to the spore (Bar = 10  $\mu$ m).

**Other material examined:**

On *Taeniatherum crinitum* (Schreb.) Nevski, Iraq, Zawita, 1 Aug. 1955, leg. V. C. Robertson (PUR F17567).

Spermogonia and aecia unknown. Uredinia mostly on adaxial surface, scattered, elliptic,

yellow or brownish yellow; urediniospores (22) 24-28  $\times$  20-23  $\mu$ m, subglobose, broadly ellipsoid or ellipsoid, wall 1.5-2  $\mu$ m, hyaline, yellowish or rarely very pale brownish, echinulate, germ pores (10)11-14 (15) scattered. Telia mostly abaxial, covered by

the epidermis, blackish, oblong, scattered or irregularly aggregated, mostly loculated with brown paraphyses; teliospores 46-72 × 16-25 μm, mostly oblong-clavate or oblong, apex rounded, truncate, conical or obliquely conical, constricted at the septum, attenuated below, wall of upper cell 1.5-2 μm at sides, thickened up to 5-8(10) μm at the apex, smooth, chestnut-brown above, golden basally; one-celled spores occasional; pedicel short, yellowish, with brown, mostly 1.5 (2) μm thick septum at the point of attachment.

Combination of morphological features and ITS sequence data separate *Puccinia taeniatheri* sp. nov., from other reported *Puccinia* species on Poaceae.

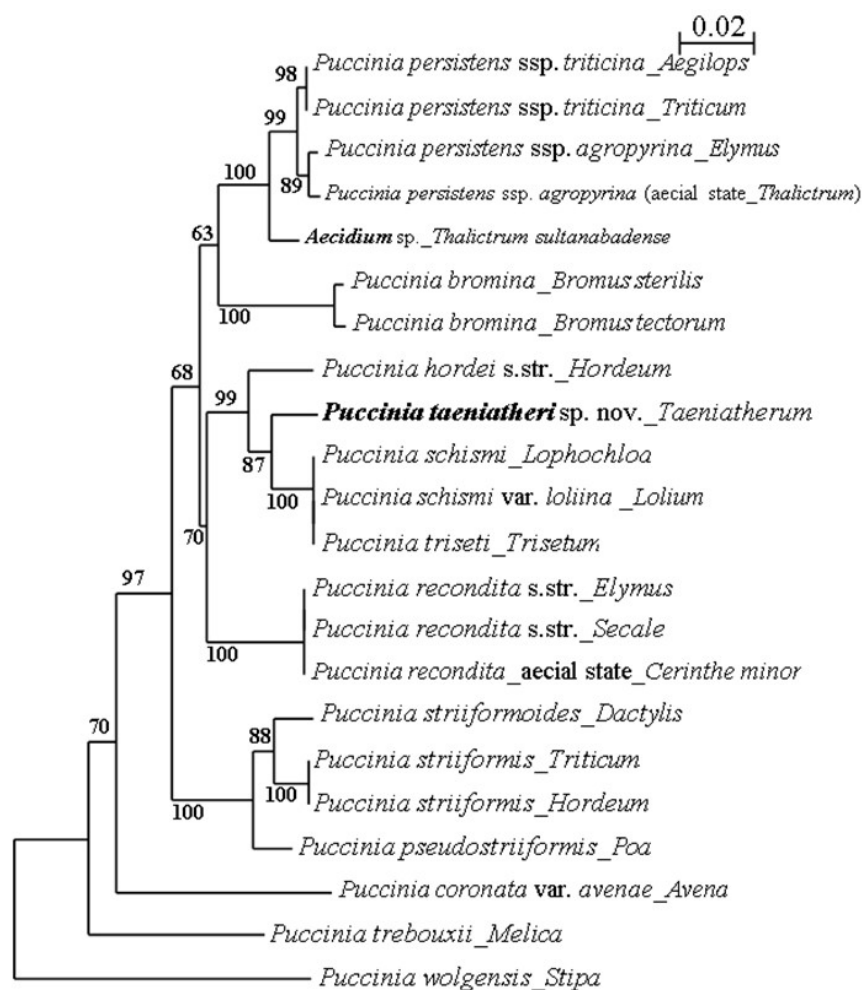
Judging from the literature (Cummins 1971; Ulyanishchev 1978; Abbasi 2002) and online databases of U.S. National Fungus Collections (<https://nt.ars-grin.gov/fungalatabases/>) the following *Puccinia* species viz. *Puccinia agropyrina* Erikss. (= *P. persistens* subsp. *agropyrina* (Erikss.) Z. Urb. & J. Marková), *P. graminis* Pers., *P. hordei* G. H. Oth, *P. rubigovera* (DC.) G. Winter (= *P. recondita* Roberge ex Desm.) and *P. striiformis* Westend., have been reported on *Taeniatherum* spp. across the world.

The molecular analysis and NJ tree reconstruction based on rDNA ITS sequencing showed that *Puccinia taeniatheri* sp. nov., differs from all above mentioned *Puccinia* species (Fig. 2). BLAST query of *P. taeniatheri* sp. nov., sequence shared 94% (660/699) identity with *P. hordei*, 94% identity (660/701) with *P. schismi*, 92% identity (642/701) with *P. recondita* and 91% identity (646/709) with *P. persistens* ssp. *agropyrina*. Comparison of *P. taeniatheri* sp. nov. ITS sequence with that obtained from *P. striiformis* using Blast 2 sequences (bl2seq) available at NCBI website also showed 89% (622/700) identity. In addition to molecular analysis, morphological data also showed that rust species on *Taeniatherum* is a new species different from all other *Puccinia* species reported on this host plant. Urediniospores

with hyaline or yellowish wall and up to 15 scattered germ pores, teliospores with brown septum at the pedicel attachment point and thin side wall of teliospore upper cell (1.5-2 μm), not thickened toward spore apex, are among characteristic features of *P. taeniatheri* sp. nov. The following identification key separates *P. taeniatheri* sp. nov., from all other *Puccinia* species reported on the genus *Taeniatherum*:

- 1-telia exposed, urediniospores with mostly 4-5 equatorial germ pores.....*P. graminis*
- 1-telia covered, urediniospores with scattered germ pores.....2
- 2-uredinia in chlorotic streaks.....*P. striiformis*
- 2-uredinia not in such streaks.....3
- 3-urediniospore wall brownish.....4
- 3-urediniospore wall hyaline to yellowish.....5
- 4-urediniospores with 6-9 scattered germ pores, teliospore pedicels do not possess a conspicuous deposition of dark brown material on the wall of the pedicel below the point of attachment.....*P. recondita*
- 4-urediniospores with 8-11 scattered germ pores, teliospore pedicels with a deposition of a brown material in form of truncate cone below the point of the spore attachment .....*P. persistens* subsp. *agropyrina*
- 5-urediniospores with 7-10 scattered germ pores, 1-celled teliospores (mesospores) abundant, the side wall of teliospore upper cell thickening progressively toward the apex.....*P. hordei*
- 5-urediniospores with 10-15 scattered germ pores, 1-celled teliospores occasional, the side wall of teliospore upper cell not thickening progressively toward the apex.....***P. taeniatheri***

The ITS sequence analysis showed that *P. hordei* with 94% identity, is among the closest species to the *P. taeniatheri* sp. nov. There is a report of *P. hordei* on *T. crinitum* from Iraq (Mathur, 1972). Re-examination of the specimen of *P. hordei* on *T. crinitum* (PUR F17567), collected from Zawita, Iraq revealed that specimen fitted well description of *P. taeniatheri* sp. nov., and was considered under the name of this taxon.



**Figure 2** Unrooted neighbor-joining tree of 22 sequences of the internal transcribed spacer (ITS) region of ribosomal DNA from graminicolous *Puccinia* species and their related Aecial states on miscellaneous plants. Bootstrap values were determined on the basis of 1000 bootstrap replications. The ITS sequences of *P. coronata* var. *avenae*, *P. trebouxii* and *P. wolgensis* were used as outgroup.

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## *Puccinia taeniatheri* یک زنگ جدید روی گندمیان از ایران

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**چکیده:** گونه زنگ *Puccinia taeniatheri* sp. nov. برای اولین بار از ایران روی گونه گیاهی *Taeniatherum asperum* از تیره گندمیان (Poaceae) براساس مطالعات ریخت‌شناسی و توالی‌یابی ناحیه ITS دی‌ان‌آر ریبوزومی توصیف می‌شود. کلید شناسایی برای گونه‌های *Puccinia* گزارش شده روی جنس *Taeniatherum* ارائه می‌گردد. همچنین بازنگری نمونه هرباریومی مربوط به گونه زنگ *P. hordei* روی *T. crinitum* گزارش شده از عراق مشخص نمود که این نمونه نیز به گونه جدید *P. taeniatheri* تعلق دارد.

**واژگان کلیدی:** Poaceae, Pucciniales, تاکسونومی، تنوع زیستی، گونه جدید