Uromyces hawksworthii nom. nov. for Aecidium goyazense, on Phthirusa stelis (Loranthaceae) from the Brazilian Cerrado

Érica S.C. Souza¹, Zuleide M. Chaves¹, William R.O. Soares¹, Danilo B. Pinho¹, and José C. Dianese¹

¹Departamento de Fitopatologia, Instituto de Ciências Biológicas, Universidade de Brasília, 70910-900 Brasília, DF, Brazil; corresponding author e-mail: jcarmine@gmail.com

Abstract: The sexual morph of Aecidium goyazense collected in the Brazilian Cerrado was morphologically characterized by light microscopy and SEM, and shown to be a species of Uromyces, for which the name Uromyces hawksworthii nom. nov. is introduced, and designated as its epitype. This is the second Uromyces species known to infect the tropical genus Phthirusa (Loranthaceae). DNA sequences were generated from the ITS and 28S rRNA (LSU) regions of DNA recovered from aeciospores as well as teliospores. This fungus is compared with other Uromyces species known from Loranthaceae.

Key words: Basidiomycota
Neotropical fungi
Pucciniaceae
Pucciniomycotina
rust fungi
Urediniomycetes

INTRODUCTION

Hennen et al. (2005) catalogued the rust fungi on Loranthaceae in Brazil, including Aecidium goyazense, Uromyces circumscriptionis, U. loranthei, and U. urbanianus. Perdomo-Sánchez & Piepenbring (2014) revised the Uromyces species known from Loranthaceae, namely, U. euphebius, U. evastigatus, U. loranthei, U. nilagiricus, U. ornatipes, U. phthirusae, U. socius, and U. urbanianus, adding two new taxa, U. bahiensis from Brazil, and U. struthanthei from Panama. They omitted A. goyazense as it was known only as an aecial morph without a connection to a telial stage. The telial stage proves morphologically to belong to Uromyces, and this is described and illustrated here, and also characterized by analysis of DNA sequences to provide a barcode for identification of the species.

MATERIALS & METHODS

Leaves of Phthirusa stelis with a gall rust were collected in Brasilia, Distrito Federal. The brown galls were covered in cylindrical to conical/subulate pale yellow aecia, and erumpent dark brown telia covered by a layer of dark brown spores. Aecidia and telia were sectioned at 15–20 µm thickness with a Micron freezermicrotome. Scale preparations of aecia, aeciospores, and teliospores from the galls were examined microscopically by Nomarski differential interference contrast under a Leica DM 2500 microscope coupled with a Leica DFC 490 digital camera; image capture and measurements were made with Leica QWin V3 software. Some samples were stained with lacto-glycerol Cotton blue and the slides sealed with nail polish. A minimum of 25 replicates of aecial and aecial structural cells were measured. Portions of dried galls with aecia and telia were fixed onto 10 mm diam copper stubs using double-sided carbon tape, and coated with gold at 25 mA, 1.10-2 mbar, for 2.5 min. for examination with a JEOL JSM-700 1F Model scanning electron microscope. Voucher specimens are deposited in the Mycological Collection of the Universidade de Brasilia (UB).

DNA extraction, PCR amplification, and DNA sequencing

To obtain spores and prevent contamination by other fungi, sori were examined under a stereomicroscope. Aeciospore and teliospore masses were removed with a needle, and placed separately in micro-centrifuge tubes (1.5 mL) stored at –20°C. Tissue samples were frozen with liquid nitrogen and ground into a fine powder with a micro-centrifuge tube pestle. DNA extraction followed the standard CTAB (cetyltrimethyl ammonium bromide) procedure (Doyle & Doyle 1990). PCRs included the following ingredients for each 25 µL reaction volume: 0.5 U Taq DNA Polymerase Platinum, 0.2 µM of each of the forward and reverse primers; plus a maximum of 10 ng/µL of genomic DNA; nuclease-free water completed the total volume. Primers ITS4-rust and ITS5-u were used to amplify the internal transcribed spacer region (ITS) of the rDNA (Pfunder et al. 2001). The LSU was amplified with a primer pair, Rust2inv and LR6 (Aime 2006, Vilgalys & Hester 1990), while LR0R and Rust1 (Moncalvo et al. 1995, Kropp et al. 1997) were used as internal sequence primers. The thermal cycle consisted of 94 °C for 4 min, followed by 30 cycles of 94 °C for 1 min (denaturation), 54 °C for 1 min (annealing), 72 °C for 1 min (elongation), and 72 °C for 5 min (final extension). PCR products were analyzed by 1% agarose electrophoresis.
gels stained with ethidium bromide in a 1X TAE buffer and visualized under UV light to check for amplification size and purity. PCR products were treated using ExoSAP-IT® (USB) and sequenced in an Applied Biosystems (ABI3130xl Model) apparatus at the Catholic University of Brasília.

The nucleotide sequences were edited with BioEdit software (Hall 2012). All sequences were checked manually, and nucleotides with ambiguous positions were clarified by both primer direction sequences. New sequences were deposited in GenBank (http://www.ncbi.nlm.nih.gov/genbank/) (Table 1).

### PHYLOGENETIC ANALYSIS

Consensus sequences were compared against GenBank’s database using Mega BLAST. Based on the BLASTn results, sequences were selected for the greatest similarity, and data from recent phylogenetic studies focused on Pucciniaceae (Bruns et al. 1992, Maier et al. 2003, Chung et al. 2004, Aime 2006, Matheny et al. 2006, Henricot et al. 2007, Maier et al. 2007, Yun et al. 2010, Dixon et al. 2010, Deadman et al. 2011, Zuluaga et al. 2011, Busby et al. 2012, McTaggart 2014, Padamsee and McKenzie 2014, Liu et al. 2015). After selection, the sequences were downloaded in FASTA format and aligned by the multiple sequence alignment program MUSCLE® (Edgar 2004), built in MEGA v. 6 software (Tamura et al. 2011). Alignments were checked and manual adjustments were made when necessary. Gaps were treated as missing data. The resulting alignment was deposited into TreeBASE (http://www.treebase.org/), accession no. 17667. Bayesian inference (BI) analysis employing a Markov Chain Monte Carlo method was performed only with LSU sequences. Before launching the BI, the best nucleotide substitution model was determined with MrMODELTEST 2.3 (Posada & Buckley 2004). Once the likelihood scores were calculated, the models were selected according to the Akaike Information Criterion (AIC).

### Table 1. GenBank accession numbers of Uromyces hawksworthii, and of all other species included in the study.

| Species                      | GenBank accession numbers (LSU)* | Source and Country                      |
|------------------------------|----------------------------------|----------------------------------------|
| Uromyces hawksworthii UB22382 | KR821139                         | Present study, Brazil                  |
| Uromyces hawksworthii UB22875 | KR821140                         | Present study, Brazil                  |
| Cumminsiella mirabilissima    | DQ354531                         | Aime (2006) Germany                    |
| Cumminsiella mirabilissima    | AF426206                         | Maier et al. (2003) Germany            |
| Puccinia coronata             | DQ354526                         | Aime (2006) USA                        |
| Puccinia coronata             | EU851141                         | Zuluaga et al. (2011)                  |
| Puccinia graminis             | AF522177                         | Bruns et al. (1992)                    |
| Puccinia graminis             | HQ412648                         | Deadman et al. (2011)                  |
| Puccinia hemerocallidis       | GU058020                         | Dixon et al. (2010) USA                |
| Puccinia hemerocallidis       | DQ354519                         | Aime (2006) USA                        |
| Puccinia heucherae            | DQ359701                         | Henricot et al. (2007) UK              |
| Puccinia heucherae            | DQ359702                         | Henricot et al. (2007) UK              |
| Puccinia hordei               | DQ354527                         | Aime (2006) USA                        |
| Puccinia melanocephala        | KP201838                         | Wang et al. Unpubl. (2014) China       |
| Puccinia melanocephala        | KP201839                         | Wang et al. Unpubl. (2014) China       |
| Puccinia nakanishiki          | GU058002                         | Dixon et al. (2010) USA                |
| Puccinia peperomiae           | EU851146                         | Zuluaga et al. (2011) Colombia         |
| Uromyces acuminatus           | GU109282                         | Yun et al. (2010) England              |
| Uromyces appendiculatus       | KM249870                        | McTaggart (2014) Australia             |
| Uromyces appendiculatus       | AY745704                         | Matheny et al. Unpubl. (2005)          |
| Uromyces ari-triphylli        | DQ354529                         | Aime (2006) USA                        |
| Uromyces ixiae                | DQ917738                         | Maier et al. (2007) South Africa       |
| Uromyces ixiae                | DQ917739                         | Maier et al. (2007) South Africa       |
| Uromyces pisi                 | AF426201                         | Maier et al. (2003) Central Eur.       |
| Uromyces striatus             | HQ412652                         | Deadman et al. (2011) Oman             |
| Uromyces striatus             | HQ317512                         | Liu et al. (2015) Canada               |
| Uromyces trifoli              | GU936634                         | Zuluaga et al. (2011) Colombia         |
| Uromyces viciae-fabae         | KJ716343                         | Padamsee & McKenzie (2014) New Zealand  |
| Uromyces viciae-fabae         | AF426199                         | Maier et al. (2003) Central Eur.       |
| Uromyces vignae               | AB115649                         | Chung et al. (2004) Japan              |
| Melampsora larici-populina (outgroup) | JQ042250 | Busby et al. (2012) USA |

* LSU: rDNA large subunit
The general time-reversible model of evolution including estimation of invariable sites and assuming a discrete gamma distribution with six rate categories (GTR+I+G) was used. The phylogenetic analysis of the dataset was performed through the CIPRES web portal (Miller et al. 2010) using MrBayes v. 3.2 (Ronquist & Huelsenbeck 2012). Four MCMC chains were run simultaneously, starting from random trees for 10,000,000 generations. Trees were sampled every 1,000th generation for a total of 10,000 trees. The first 2500 trees were discarded as the burn-in phase of each analysis. Posterior probabilities were determined from a majority-rule consensus tree generated with the remaining 7500 trees. Trees rooted to Melampsora larici-populina were visualized by FigTree (Rambaut 2009), and exported to graphic programs.

RESULTS

Phylogeny

Amplification and sequencing of the LSU and ITS rDNA regions were successful for two specimens obtained from both the aecidial (UB22382) and telial (UB22875) morphs. The amplification of the partial 28S rDNA and ITS revealed sequences of ca. 1500 and 450 bp, respectively (Accession Numbers, LSU: UB22382=KR821139, UB22875=KR821140 and ITS: UB22382=KR821137, UB22875=KR821138). The LSU and ITS sequences obtained from aeciospores and teliospores were identical. The partial large subunit of rDNA (LSU) was selected for molecular phylogenetic identification because this molecular marker is widely informative, 211 were variable and 810 were conserved. 1037 bp of aligned positions, 97 of which were parsimony informative, 211 were variable and 810 were conserved.

TAXONOMY

Uromyces hawksworthii E.S.C. Souza, Z.M. Chaves, W.R.O. Soares, D.B. Pinho & Dianese, nom. nov. MycoBank MB812738

(Figs 1–2)

Replaced synonym: Aecidium goyazense P. Henn., Hedwigia 34: 101 (1895).

Non Uromyces goyazensis P. Henn., Hedwigia 34: 89 (1895)

Etymology: Named after David Leslie Hawksworth, Honorary President of the International Mycological Association.
Fig. 1. A–H. *Uromyces hawksworthii* (UB Mycol. Col. 22875): aecidal gall development and morphology of the aecia, A. Early stage of gall formation. B. Intermediate stage of two galls. C. Two mature galls bearing numerous aecidia. D. Cross section through a developing aecidium. E. Aecidia after aeciospore release. F. Peridium internal texture. G. Aeciospores. H. Detail view of the aeciospore wall. Bars: A–C = 2 mm, D = 50 µm, E = 300 µm, F = 20 µm, G = 10 µm, H = 1 µm.
**Key to *Uromyces* species on Loranthaceae**

1. Teliospores smooth-walled, 30–45 × 21–30 µm, distal wall to 8 µm thick .......................................................... *U. nilagiricus*
   Teliospores not smooth-walled ................................................................................................................................. 2

2. Teliospores mostly less than 40 µm long .................................................................................................................... 3
   Teliospores mostly more than 40 µm long .................................................................................................................. 7

3. Teliospores showing pedicels ornamented by conspicuous anelations ................................................................. *U. ornatipes*
   Teliospores not as above ........................................................................................................................................ 4

4. Teliospore wall reticulate-striate or reticulate ......................................................................................................... 5
   Teliospore wall not as above .................................................................................................................................. 6

5. Teliospore wall apically thickened ......................................................................................................................... *U. circumscriptus*
   Teliospore wall evenly 2 µm thick ......................................................................................................................... *U. bahiensis*

6. Teliospores smooth to finely verrucose; uredinia paraphysate, urediniospores echinulate, spines abundant ................................................................. *U. loranthis*
   Teliospores longitudinally striate; uredinia a paraphysate, urediniospores echinulate .................................................. *U. socius*

7. Teliospores short-pedicellate, pedicels to 50 µm long ................................................................................................. 8
   Teliospores long-pedicellate, pedicels reaching 90 to 160 µm long ........................................................................ 10
**DISCUSSION**

_Uromyces hawksworthii_ is morphologically different from other species reported from Brazil on _Loranthaceae_, in that it has erect cylindrical to conical or subulate aecia to 3.5 mm tall, located on well-defined hard pulvinate to subglobose brown galls. Furthermore, _U. hawksworthii_ is phylogenetically distinct from the taxa presently accommodated in GenBank. Based on a megablast search of GenBank, the closest hits using the LSU sequence are _Puccinia heucherae_ RHSS296/05 (GenBank DQ359702; Identities (98 %) = 1036/1060, _U. acuminatus_ CT-V080623-3 (GenBank GU109282; Identities (98 %) = 1035/1059, _U. aristiphylia_ U637 (GenBank DQ354529; Identities (98 %) = 1034/1057, _Puccinia graminis_ U-674 (GenBank HQ412648; Identities (98 %) = 1023/1048, and

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**Fig. 3.** Phylogenetic tree inferred from the Bayesian analysis based on the LSU sequences of _Uromyces_ and related taxa. The Bayesian posterior probability values above 0.75 are indicated at the nodes. GenBank accession numbers are in parentheses. The specimens in this study are highlighted in bold. Black squares and circles indicate DNA sequences obtained from aeciospores and teliospores, respectively. The tree was rooted to _Melampsora larici-populina._
P. hordei AFTOL-ID 1402 (GenBank DQ354527; Identities (98 %) = 1017/1043. Additionally, both aecial and telial specimens of *Uromyces hawksworthii* examined in this study were similar and formed a clade with *Uromyces ari-triphylli* and *Puccinia peperomiae* (Fig. 3). Within this strongly supported clade (posterior probability = 0.98), the two specimens of *U. hawksworthii* formed a sister clade with other taxa included. As rust fungi from South America are poorly characterized molecularly, additional DNA sequence data will be needed to further clarify the phylogeny of rust fungi from tropics.

The aecial morph of this fungus was described as *Aecidium goyazense* (Hennings 1895), but the telial morph has not been previously reported. The binomial *Uromyces goyazensis* is pre-occupied by a rust fungus found on *Bauhinia* (Hennings 1895), which means that the name *Aecidium goyazense* cannot be recombined into *Uromyces* as this would create an homonym to be rejected (Art. 53.1). Consequently, we have given the fungus the new name *Uromyces hawksworthii* here.

Two identification keys for *Uromyces* species on *Loranthaceae* are available (Hennen *et al.* 2005, Perdomo-Sánchez & Piepenbring 2014). In each key the species were separated by the shape and ornamentation of the teliospores, aecia, aeciospores, presence or absence of the uredinial phase, and host species. Perdomo-Sánchez & Piepenbring (2014) revised and illustrated by light microscopy and SEM, the type specimens of *Uromyces* on *Loranthaceae* around the world, except for *U. nilagiricus*, a species reported on *Loranthus* sp. from India, for which type material was not available. This is the only species found outside Latin America distinguished by smooth teliospores (Ramakrishnam & Ramakrishnam 1950).

Based on teliospore wall characteristics, the *Uromyces* species on *Loranthaceae* are distributed in two well-defined groups. One has superficially verrucose or markedly striate teliospores, including *U. euphrubius*, *U. ornatipes*, *U. loranthi*, *U. phthirusae*, and *U. socius* (Sydow 1920, Arthur 1915, 1918, Perdomo-Sánchez & Piepenbring 2014). The other group has teliospores with pitted to foveate surfaces, including *U. bahiensis*, *U. circumscriptus*, *U. evastigatus*, *U. loranthi*, *U. phthirusae*, *U. struthanthi*, and *U. urnabianus*. In the latter group of species, only *U. loranthi* (aecidia unknown, teliospore walls verrucose) and *U. phthirusae* (teliospore walls striate) have a known uredinial phase.

**ACKNOWLEDGEMENTS**

We thank Mariza Sanchez, Robert N.G. Miller, and Dirceu Macagnan for support and help. Thanks are also due to CNPq/Brazil for financial support through the PBBIO-Cerrado Project.

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