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ARTICLE

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

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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

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INTRODUCTION

Hennen *et al.* (2005) catalogued the rust fungi on *Loranthaceae* in Brazil, including *Aecidum goyazense*, *Uromyces circumscriptus, U. loranthi,* and *U. urbanianus*. Perdomo-Sánchez & Piepenbring (2014) revised the *Uromyces* species known from *Loranthaceae*, namely, *U. euphlebius*, *U. evastigatus*, *U. loranthi*, *U. nilagiricus*, *U. ornatipes*, *U. phthirusae*, *U. socius*, and *U. urbanianus*, adding two new taxa, *U. bahiensis* from Brazil, and *U. struthanthi* 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 freezing microtome. Squash 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 spore 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 nucleotide, 5 mL 10X buffer, 1.5 mM $MgCl_2$, 0.4 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 rRNA (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

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 nakanishikii	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

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, Padamse 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).

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 & Heulsenbeck 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 of the fungus because this molecular marker is widely recommended for genus and species level identification of all rust fungi (Hyde *et al.* 2014). The ITS sequences were lodged in GenBank and UNITE (Nilsson *et al.* 2014). Based on the results from the primary LSU data matrix (tree not shown) and the dataset for rust fungi (tree not shown) from Hyde *et al*. (2014), 31 taxa were selected from across the breadth of the LSU derived phylogenetic trees. The dataset totaled 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.

Diagnosis: A *Uromyces* species on *Phthirusa stelis* (*Loranthaceae*) with elongate aecidia on the surface of pulvinate corticoid leaf galls up to 1.2 cm diam, and characteristically long-pedicellate teliospores.

Type: **Brazil:** Goiás, Serra dos Pyreneos, on *Phthirusa stelis* [as *Loranthus* sp.], Aug. 1892, *Ule 1909* (B 2945 – holotype); Brasília, Guará I, Associação dos Criadores de Pássaros de Brasília, 15º 48´ 42.12" S × 47º 58´ 22.53" W, on leaves of *Phthirusa stelis*, 9 Feb 2014, *J. C. Dianese* (UB Mycol. Col. 22875 – **epitype designated here**, MBT 201535).

Description: *Spermogonia* not seen. *Aecidia* 5–6 mm long × 300–400 µm wide, amphigenous, mostly epiphyllous, gregarious, initially subepidermal, erumpent, cylindrical, conical/subulate, bright yellow, grouped on a light brown to brown hemisphaerical to pulvinate area, 0.5–1 cm diam before emergence of the aecidia, to 1.2 cm diam at aecidial maturity; *peridial cells* 30–(*36*)–57 × 21–(*22*)–35 µm, oblong to rhomboidal, outer wall rough, hyaline or slightly yellow. *Aecidiospores* (24–)25–29(–35) × (17–)21–25(–27.5) µm, angular, rhomboidal, subglobose, ovoid, catenulate, verrucose, hyaline to pale yellow; *walls* 2–3.5(–4.5) µm. *Uredinia* not seen. *Telia* 1–(*2*)–3 mm diam, on circular light brown spots, amphigenous, subepidermal, erumpent,, pulverulent, dark brown, amphigenous, flattened to slightly domed, aparaphysate, but showing large numbers of paraphysis-like long teliospore pedicels. *Teliospores* (34–) 39–43(–46) × (18–) 22–24 (–26.5) µm, 1-celled, oblong-ellipsoidal; wall pale to chestnut brown, reticulate, pitted in SEM, germ pores not observed; *lateral wall* 2.5–3(–4) µm thick, apical wall 5–6(–7) µm thick, long-pedicellate; *pedicels* (48–)84–143(–157) × (4–)5–6(–7) µm, seldom persistent in mature teliospores, cylindrical, smooth, thin-walled, hyaline, flexuous.

Other specimens examined (on leaves of *Phthirusa stelis*): **Brazil**: Brasília, Guará I, Associação dos Criadores de Pássaros de Brasília, 18 May 2014, *J. C. Dianese* (UB Mycol. 22879); Asa Norte, Campus Universidade de Brasília, near University Restaurant, 17 Sep. 2012, *E. S. C. de Souza* (UB Mycol. 22389); Parque Olhos D'Água, 12 Sep. 2012, *E. S. C. de Souza* (UB Mycol. 22382); Lago Sul, Brasília Botanical Garden, 23 Apr. 2012, *E. S. C. de Souza* (UB Mycol. 22184); Asa Norte, L4 Avenue, Estação Experimental de Biologia, Universidade de Brasília, 29 Sep. 2009, *M. D. M. dos Santos* (UB Mycol. 21084); Vargem Bonita, Fazenda Água Limpa, Universidade de Brasília, 12 Sep. 2007, *N. M. Toledo de Souza* (UB Mycol. 20762); Planaltina-DF, Estação Ecológica de Águas Emendadas, 11 Jun. 2007, *V. R.Rodrigues* (UB Mycol. 20651); Asa Norte, Campus Universidade de Brasília, near the Rector´s office, 9 May. 2007, *Z. M. Chaves* (UB Mycol. 20569); Super Quadra Norte 410 near the N Bloc, 18 Aug. 2003, *R. C. P. Carvalho* (UB Mycol. 19398); Brasília National Park, 27 Sep. 1995, *Z. M. Chaves* (UB Mycol. 10125).

Fig. 1. A–H. *Uromyces hawksworthii* (UB Mycol. Col. 22875): aecidial 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.

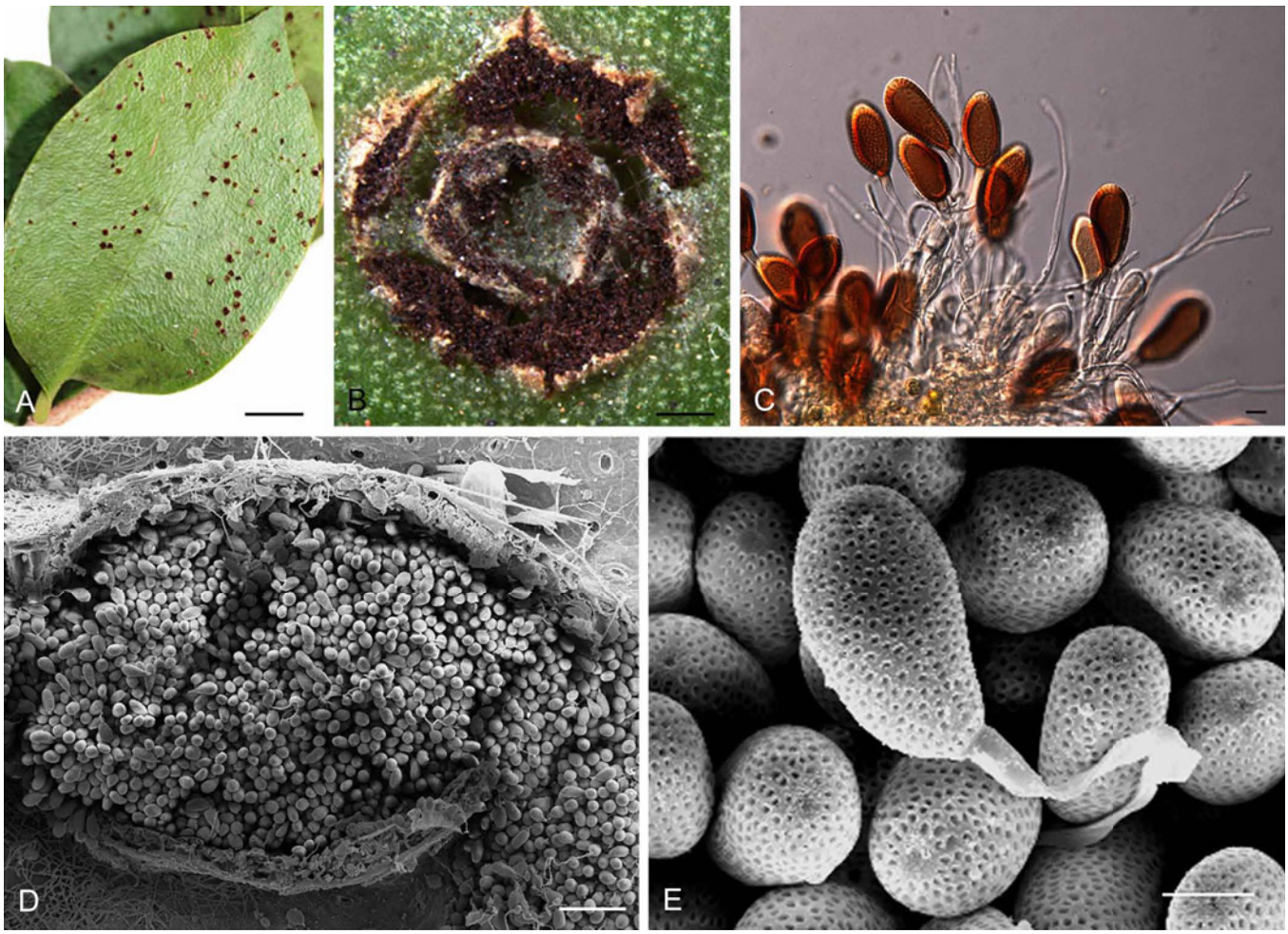

Fig. 2. A–E. *Uromyces hawksworthii* (UB Mycol. Col. 22875): **A.** Circular to irregular dark brown telia on the adaxial face. **B.** Erumpent telium. **C.** A group of mature teliospores and several long paraphysis-like pedicels. **D.** Telium seen in SEM. **E.** Teliospores showing the characteristically pitted wall in SEM. Bars: A = 1 cm, B = 2 mm, C = 10 µm, D = 100 µm, E = 5 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 (1) Teliospores mostly less than 40 µm long 3
Teliospores mostly more than 40 µm long 7

3 (2) Teliospores showing pedicels ornamented by conspicuous annelations ..... **U. ornatipes**
Teliospores not as above 4

4(3) Teliospore wall reticulate-striate or reticulate 5
Teliospore wall not as above 6

5(4) Teliospore wall apically thickened ..... **U. circumscriptus**
Teliospore wall evenly 2 µm thick ..... **U. bahiensis**

6(4) Teliospores smooth to finely verrucose; uredinia paraphysate, urediniospores echinulate, spines abundant ..... **U. loranthi**
Teliospores longitunally striate; uredinia aparaphysate, urediniospores echinulate ..... **U. socius**

7(2) Teliospores short-pedicellate, pedicels to 50 µm long 8
Teliospores long-pedicellate, pedicels reaching 90 to 160 µm long 10

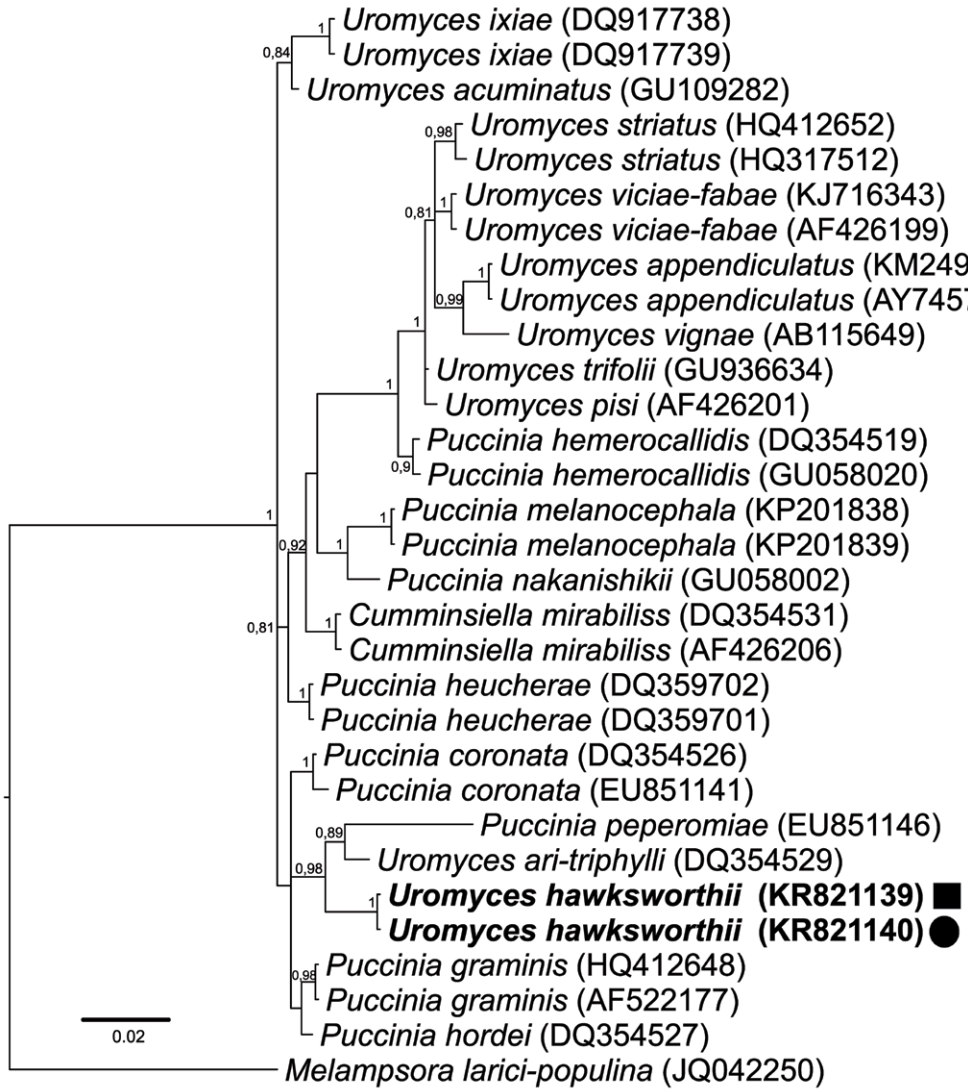

Fig. 3. Phylogenetic tree inferred from the Bayesian analysis based on the LSU sequences of *Uromyces* and related taxa. The Bayesian posterior probability values above 0.75 are indicated at the nodes. GenBank accession numbers are in parentheses. The specimens in this study are highlighted in bold. Black squares and circles indicate DNA sequences obtained from aeciospores and teliospores, respectively. The tree was rooted to *Melampsora larici-populina*.

8(7) Teliospores showing very fine ornamentations on a reticulate disposition, urediniospores coarsely reticulate; aecidiospores verrucose, subtuberculate ..... **U. phthiruzae**
Teliospores not as above, aecidiospores minutely verrucose to verrucose 9

9(8) Teliospores verrucose-striate; aecidiospores verrucose ..... **U. urbanianus**
Teliospores reticulate, aecidiospores minutely verrucose ..... **U. evastigatus**

10 (7) Teliospores reticulate-foveate; aecidiospores spinose-echinulate ..... **U. struthanthi**
Teliospores, non reticulate-foveate; aecidiospores verrucose ..... **U. hawksworthii**

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* RHS5296/05 (GenBank DQ359702; Identities (98 %) = 1036/1060, *U. acuminatus* CT-V080623-3 (GenBank GU109282; Identities (98 %) = 1035/1059, *U. ari-triphylli* U637 (GenBank DQ354529; Identities (98 %) = 1034/1057, *Puccinia graminis* U-674 (GenBank HQ412648; Identities (98 %) = 1023/1048, and

P. hordei AFTOL-ID 1402 (GenBank DQ354527; Identities (98 %) = 1017/1043. Additionally, both aecidial 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 aecidial 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. euphlebius*, *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.

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