

Uredinia and further morphological and molecular confirmation of the rust fungus *Uromyces hawksworthii* on *Loranthaceae* from the Brazilian Cerrado

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Abstract: This study describes *Uromyces hawksworthii* (*Basidiomycota: Pucciniales*) on morphological and molecular level. This rust fungus often infects *Phthirusa stelis* (*Loranthaceae*), a common hemiparasite on trees in the Brazilian cerrado. The morphology of the rust was evaluated by light and scanning electron microscopy. Partial sequences of the ITS region of rDNA were obtained from both aecial and telial stages using cloning techniques. Morphological features of the aecial stage match the description of *Aecidium goyazense*, while analysis of urediniospores and teliospores indicates that the correct name for this fungus is *Uromyces hawksworthii*. Molecular identification and maximum likelihood analysis revealed that this species belongs to the *Pucciniaceae*, which contains the *Puccinia/Uromyces* genus complex. Furthermore, four sequences of aeciospores and two of the teliospores showed more than 99% of similarity compared with each other. Morphological and genetic studies confirmed that *Uromyces hawksworthii* is a macrocyclic autoecious rust fungus, whose aecial stage is *A. goyazense*, as has already been found by SOUZA & al. 2015.

Zusammenfassung: Die vorliegende Untersuchung beschreibt *Uromyces hawksworthii* (*Basidiomycota: Pucciniales*) morphologisch und molekular. Dieser Rostpilz infiziert häufig *Phthirusa stelis* (*Loranthaceae*), einen verbreiteten Hemiparasiten auf Bäumen im brasilianischen Cerrado. Die Morphologie des Rostpilzes wurde licht- und rasterelektronenmikroskopisch untersucht. Aus Aecien und Telien wurden Teile der ITS-Region der rDNA sequenziert unter Verwendung von Klonierungstechniken. Die morphologischen Merkmale der Aecien entsprechen der Beschreibung von *Aecidium goya-*

zense, während die Analyse der Urediniosporen und Teliosporen zeigt, dass der korrekte Name für diesen Pilz *Uromyces hawksworthii* ist. Die molekulare Identifikation und Maximum-Likelihood-Analyse ergab, dass diese Art zur Familie *Pucciniaceae* gehört, die den *Puccinia/Uromyces* Gattungskomplex enthält. Miteinander verglichen zeigten darüber hinaus vier Sequenzen der Aeeciosporen und zwei der Teliosporen mehr als 99% Ähnlichkeit. Sowohl die morphologische als auch die genetische Analyse bestätigen somit, dass *Uromyces hawksworthii* ein makrozyklischer autözischer Rostpilz ist, dessen Aecien als *Aecidium goyazense* beschrieben wurden, wie bereits von SOUZA & al. 2015 festgestellt wurde.

Loranthaceae is a ubiquitous family of flowering parasitic plants that can be found in the majority of tropical and subtropical areas worldwide. These plants are epiphytic hemiparasites that adhere to the branches and twigs of trees (WALLY & al. 2012). There are 73 genera, which contain more than 900 species of hemiparasitic shrubs (VIDAL-RUSSEL & NICKRENT 2008).

Nine *Uromyces* species have been identified on *Loranthaceae*, namely *Uromyces ornatipes* ARTHUR, *U. euphlebius* SYD. & P. SYD., *U. socius* ARTHUR & HOLW., *U. loranthis* H. S. JACKS. & HOLW., *U. phtirusae* MAYOR, *U. circumscriptus* NEGER, *U. urbanianus* HENN., *U. evastigatus* CUMMINS, and *U. hawksworthii* E. S. C. SOUZA, Z. M. CHAVES, W. R. O. SOARES, D. B. PINHO & DIANESE (SYDOW & SYDOW 1910, CUMMINS 1939, SOUZA & al. 2015). *Uromyces loranthis* was originally described as *Puccinia loranthis* by the early Italian-Argentinian botanist, SPEGAZZINI, in 1886 (SACCARDO 1888). CUMMINS (1939) reported *U. evastigatus* as a new species after it had previously been described as *U. urbanianus* P. HENN. and *Pucciniola urbaniana* ARTHUR. Thus, the characterization of several species using finely reticulate or verrucose teliospores needs to be re-defined based on morphology.

Except *U. hawksworthii*, the eight *Uromyces* species listed above are in the CUMMINS' key (HENNEN & al. 2005). This includes *U. neophthirusae* JACKSON as the new (invalidly published) name for *U. phtirusae*, which often occurs in *Loranthus* in Brazil. In *Pucciniales* occurring in Colombia, the name *U. neophthirusae* was maintained, synonymous with *U. phtirusae*, which is commonly found on *Phthirusa pyrifolia* (BURITICÁ & PARDO-CARDONA 1996). SÁNCHEZ & PIEPENBRING (2014) reported two new species in *Loranthaceae* based on herbarium material. These were *U. bahiensis* PERD-SANCH. found on undetermined *Loranthaceae* and *U. struthanti* PERD-SANCH. found on *Struthanthus* (*Loranthaceae*). In addition, they published a key of *Uromyces* species within this family. The eight species originally in the CUMMINS' key were maintained and *U. milagiricus* T. S. RAMAKR. & K. RAMAKR, which was identified in 1950 on *Loranthus* sp. in India, was included, in addition to the two new *Uromyces* species. Spermogonia, uredinia, basidia or basidiospores were not found in either of the new species (BERNDT 2002). The description of these species was based on morphological features of different rust fungi stages using optical and scanning electron microscopes (SEM). There was no evidence of a connection between the aecial and uredinial/telial stages by molecular analysis.

A comparative study of all *Aecidium* and *Uromyces* species found on *Loranthaceae* is necessary because in several fungal species, the urediniospores were wrongly identified as teliospores. For example, for *U. phtirusae* on *P. pyrifolia*, the fact that teliospores in this species were minutely verrucose was overlooked, whereas the striate urediniospores had four distinct equatorial pores (JACKSON 1927). Aecial stages of *bulbifaciens* NEGER, *A. cookeanum* DE TONI., *A. luculentum* SYD. & P. SYD., *A.*

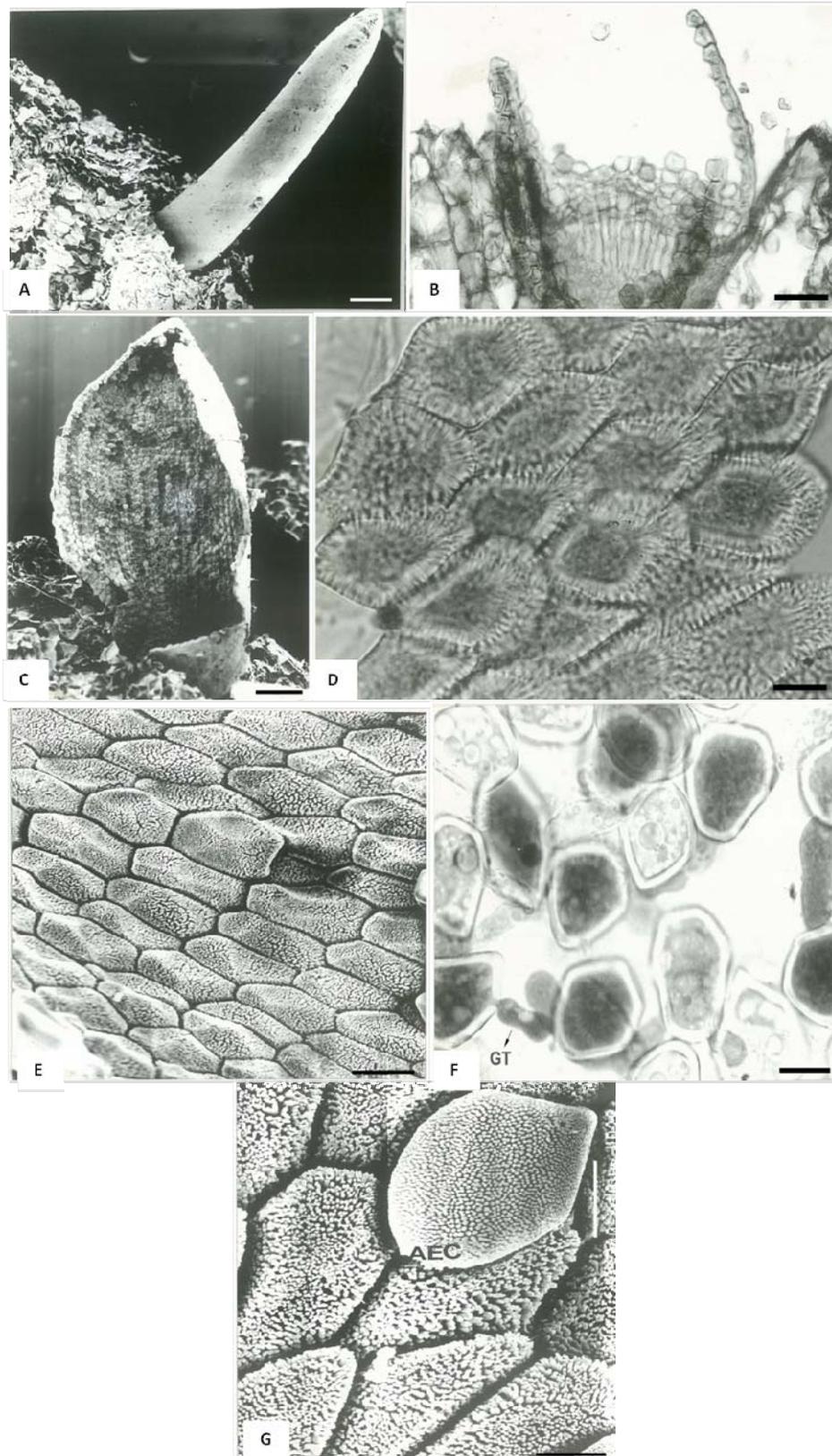


Fig. 1. Aerial stage of *U. hawksworthii* under the scanning electron (SEM) and light microscope (LM). *A* Aecium in one gall under SEM. *B* Cut of aecium showing column of aeciospores and peridium under the LM. *C* Open aecium showing internal wall, peridial cells and aeciospores under SEM. *D*, *E* Rhomboid and verrucose peridial cells of aecium under LM and SEM respectively. *F* Germinated aeciospores showing germ tubes (GT) (arrow). *G* One aeciospore (AEC) over peridial cells under SEM. Bars: *A* = 180 μ m, *B* = 120 μ m, *C* = 40 μ m, *D* = 150 μ m, *E*, *G* = 50 μ m, *F* = 70 μ m.

phrygilanthi H. S. JACKS & HOLW., *A. struthanthi* H. S. JACKS. & HOLW. (JACKSON 1927), and *A. goyazense* HENN. on *Phthirusa stelis* in Goiás, Brazil (SACCARDO 1899, SYDOW & SYDOW 1924). Recently, SOUZA & al. (2015) reported *Uromyces hawksworthii* SOUZA, E. S. C, CHAVES, Z. M. C., SOARES, W. R. O., PINHO, D. B. & DIANESE, J. C. as replacement name for *A. goyazense* on *Phthirusa stelis* (*Loranthaceae*), based on DNA sequences generated from the ITS and 28S rRNA (LSU) regions of DNA recovered from aeciospores and teliospores.

The current study aims to describe and confirm the morphological characteristics of aecial and uredinial/telial stages of the rust fungus on *P. stelis* by using microscopy (optical and SEM), and to compare both stages by conducting molecular analyses of this rust species on *Loranthaceae*.

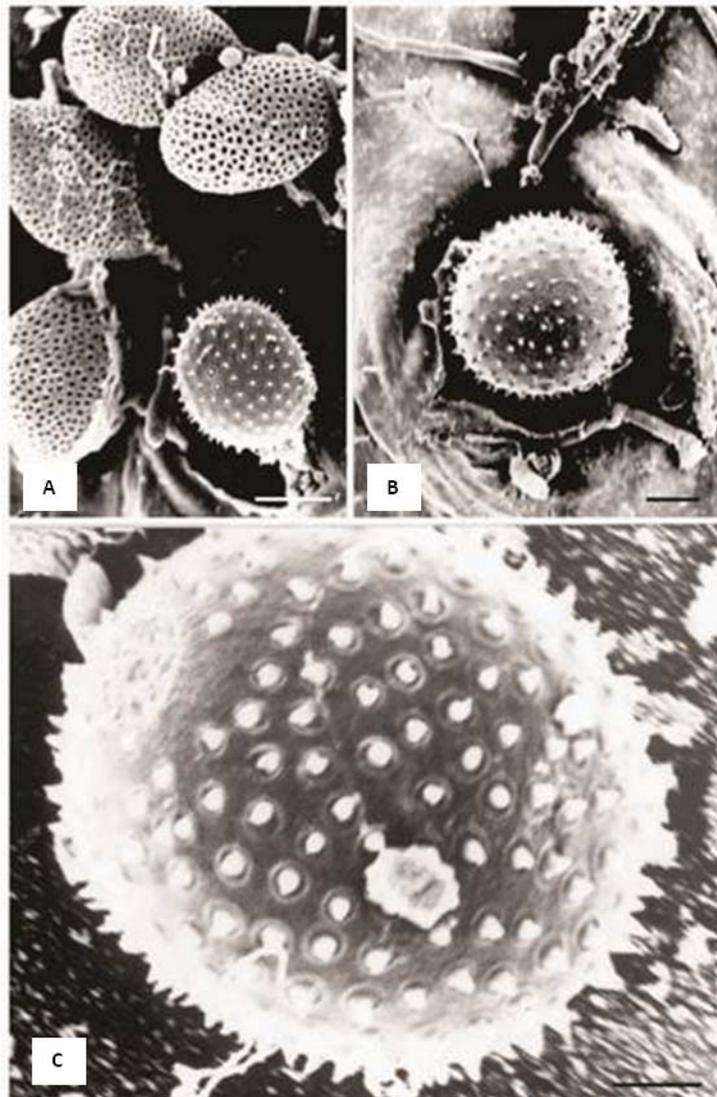


Fig. 2. Uredinial stage of *U. hawksworthii*. A, B Substomatal uredinia showing globose, echinulate and pedicellate urediniospores getting out one by one from stomata under SEM. C Urediniospores showing details of echinulations under the SEM. Bars: A = 10 μ m, B, C = 5 μ m.

Materials and methods

Specimens investigated

Specimens were collected from various regions in the federal district of Brazil. They were isolated from the living leaves of *P. stelis* at different times, between 1992 and 2003. The specific regions and

collection dates are as follows: Brasília, (Embrapa), CPAC Planaltina, DF, August 21, 1997, D. V. REZENDE 39 (UB col. micol. 15138). Brasília, Península Norte, QI 04, Cj 06, It 22, May 5, 1992, J. C. DIANESE 400 (UB col. micol. 1482, 1483, 1484, 1485, 1486, 1487, 1488, 1489). Brasília, Península Norte, Cj 06, It 22, August 9, 1992, J. C. DIANESE 406 (UB col. micol. 1513, 1514, 1515, 1516, 1517, 1518, 1519, 1520, 1521, 1522, 1523, 1524, 1525, 1526, 1527, 1528, 1529, 1530). Brasília, Península Norte, November 11, 1992; J. C. DIANESE (UB col. micol. 2761). Brasília, Fazenda Água Limpa, UnB, February 19, 1993, R. B. MEDEIROS (UB col. micol. 3151). Planaltina 166, Estação Ecológica Águas Emendadas, February 19, 1994, C. A. INÁCIO (UB col. micol. 5955). Brasília, Embrapa, CPAC, Planaltina, DF, August 21 1997. D. V. REZENDE (UB col. micol. 15139). Brasília, DF, SQN 410, Bloco N, August 18, 2003, R. C. PEREIRA (UB col. micol. 19398).

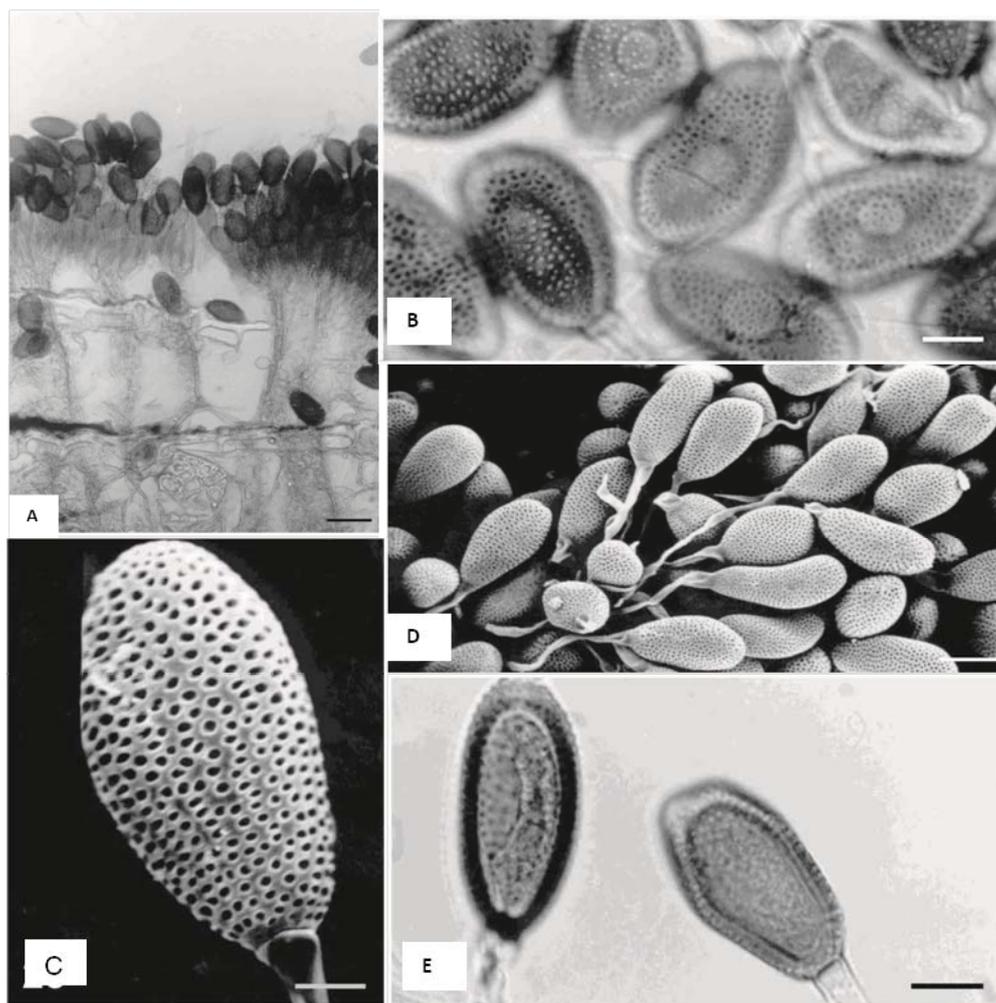


Fig. 3. Telial stage of *U. hawksworthii*, A Cut of telia under LM showing spores and fungus mycelia on internal leaf tissue B, D Reticulate, pitted, obovoid to elliptical teliospores under LM and SEM showing long persistent pedicells. C Details of teliospore pitted under the SEM. E Pedicellate teliospores showing papillae on the top under LM. Bars: A = 50 μ m, B = 30 μ m, C = 20 μ m, D = 10 μ m, E = 5 μ m.

Morphological characterization

Phthirusa stelis is an obligate parasite commonly found on trees. As a result, pathogenicity tests by spore inoculation are theoretically very difficult. Plant leaves containing aecial galls and uredinia/telia of rust were deposited in the Mycological Reference Collection of the University of Brasília (CRMUnB). Leaf sections (7 \times 7 mm) were cut using a freezing microtome (Micron HM505E). These samples were stained with lacto-glycerol-cotton blue or glycerol-KOH-phloxine B, and the slides were sealed using nail polish, labeled, and stored for subsequent analysis. They were then observed and imaged using an optical microscope (Zeiss-Axiophot E, Oberkochen, Germany) at 400 \times magnifica-

tion. Size measurements were performed in at least 20 aeciospores and 50 teliospores. Leaf sections containing uredinia/telia and aecial galls were fixed with 0.1 M sodium cacodylate buffer, pH 7.4, and 2% glutaraldehyde (v/v) for 24 h. They were then washed five times with the same buffer solution before being treated with 1% osmium tetroxide (OsO₄) in cacodylate buffer at 4°C for 4 h. The samples were washed as described with the buffer solution and dehydrated for 15 min in an aqueous series with increasing concentrations of acetone (15, 30, 50, 75 and 100%). The material was critical-point-dried using a Balzers CPD-030 (Balzers, Fürstentum Liechtenstein) with liquid CO₂. Dried tissue fragments were placed on metal cubes coated with silver conductor paste. The samples were sputter coated with gold using Balzers SCD50. Subsequently, the samples were observed and imaged using a SEM (Jeol -JSM 840-AE, Japan), with 5 and 10 KV of acceleration and a working distance of 20 mm. The fungal structures were measured using the scale bars on each image.

Molecular identification (DNA extraction, Polymerase Chain Reaction, Cloning, and sequencing)

DNA was isolated from frozen spores of the aecial (1 template) and telial (2 templates) stages of the fungi using the DNAeasy Plant Mini Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. The DNA template was checked by electrophoresis before polymerase chain reaction (PCR) was performed. A part of the internal transcribed spacer (ITS) region was amplified using the oligonucleotide primers ITS5-u (5'-caaggtttctgtaggtg-3') and ITS4-u (5'-ggctttccctcttcat-3') (PFUNDER & al. 2001), which are more suitable for amplifying the genomic DNA of *Pucciniaceae* than other known primers. PCR mixtures (25 µl) contained 0.5 µl template DNA, 12.5 µl of 2X KAPA2G Robust HotStart Ready Mix (Nippon Genetics, Tokyo, Japan), and 6 µl of each oligonucleotide, forward and reverse (1 µM). The PCR conditions were as follows: pre-denaturation for 2 min at 94°C, followed by 35 cycles of 1 min at 94°C, 10 s at 55°C, and 15 s at 72°C, with final extension for 10 min at 72°C. Amplification products were separated on a 1.0% agarose gel and detected by ethidium bromide staining and UV illumination.

The PCR products obtained were gel-purified using the Wizard SV Gel and PCR Clean-up System (Promega, Tokyo, Japan), and then ligated with the TA-cloning vector pMD20 (Takara, Tokyo, Japan) using the Mighty Mix DNA Ligation Kit (Takara, Tokyo, Japan). Subsequently, the reaction mixture was transformed into competent *Escherichia coli* DH5α Electro-Cells (Takara, Kyoto, Japan) by electroporation.

From the transformants, the plasmid DNA was purified with the FastGene Plasmid Mini kit (Nippon Genetics, Tokyo, Japan) and checked for the insert by PCR using SP6 and M13-20 primer pairs. A total of six plasmid samples from two clones of each template were sequenced by Fasmac (Japan) using the SP6 oligonucleotide.

The six analyzed clones, four from the aecial and two from telial stages, were sequenced, and the results were compared to homologous sequences registered in GenBank (<http://www.ncbi.nlm.nih.gov>) using the standard nucleotide BLAST protocol. Subsequently, the sequences were scanned for similarity with those in the databank. These sequences were then manually aligned with groups representing fungal lineages to obtain the approximate identification of the pathogenic fungi. The reference sequence and the out-group (*Melampsorium betulinum*) from the GeneBank are presented in FASTA format. Alignment of DNA sequences and the phylogeny tree were performed using the Phylogeny feature of the MEGA 6.06 program with 1000 bootstrap replications, and Maximum Parsimony analysis of taxa and evolutionary distance grouping were performed according to the Kimura model. The sequences from both aecial and telial stages were aligned for comparison.

All sequences from the six clones were registered in the DNA Data Bank of Japan (DDBJ) (<http://www.ddbj.nig.ac.jp>) with the following registration numbers: Clone 1 - LC050417; Clone 2 - LC050418; Clone 3 - LC050419; Clone 4 - LC050416; Clone 5 - LC050415; Clone 6 - LC050414. All were registered as *U. hawksworthii*.

Results

Uromyces hawksworthii E. S. C. SOUZA, Z. M. CHAVES, W. R. O. SOARES, D. B. PINHO & DIANESE (SOUZA & al. 2015) (Figs. 1–4)

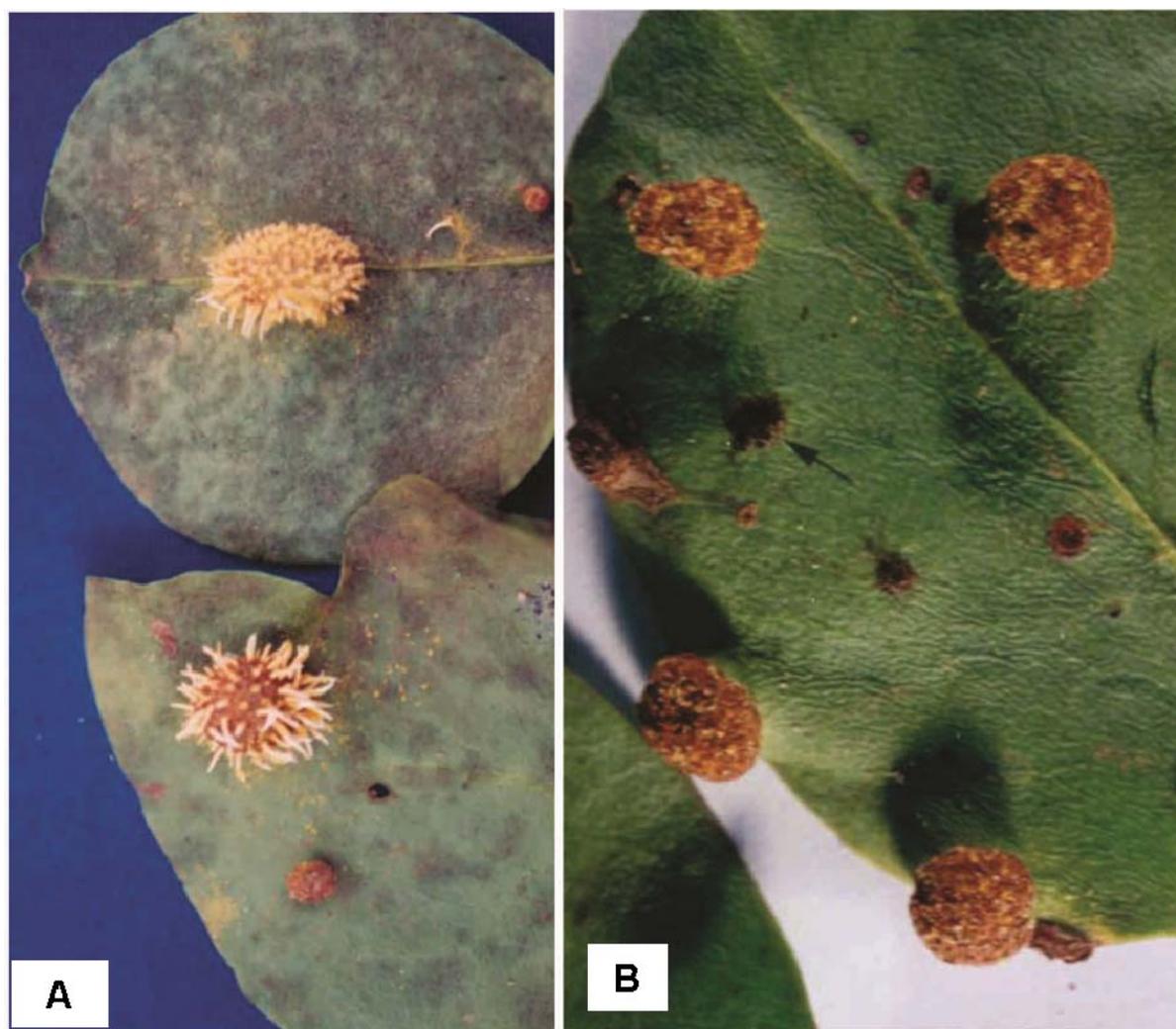


Fig. 4. Aecial and telial stages of *U. hawksworthii* on mature leaves of *Phthirusa stelis* under LM. A Galls of the rust fungus covered by tubular white to yellowish aecia. B Old galls and telia (arrow) in the same leaf.

Morphological characterization

Spermatogonia: not detected.

Aecidia: amphigenous, abundantly present on *P. stelis* leaves, hard deformed galls of (0.4–)0.7(–3) × (0.4–)0.5(–0.7) cm, long with well-developed peridia, tube shaped [(0.8–)1.3(–1.7) × (0.23–)0.3(–0.4) mm], yellow, yellowish or white, erect (Fig. 1A–G). Peridial cell conjuncts rhomboid (32–)42(–50) × (18–)21(–27) μm, external wall striate, 3–5 μm thick, internal wall verrucose, 2 μm thick (Fig. 1D–G).

Aeciospores: angular-globose, oval or ellipsoid, dense minutely verrucose, yellow, greenish, or sub-hyaline, (24–)27(–34) × (22–)25(–30) μm, wall 0.8–1 μm thick (Fig. 1F, G).

Uredinia: amphigenous substomatal, producing initially pedicellate urediniospores one by one and the uredinia seen together with telia (Fig. 2 A, B).

Urediniospores: (20–)21(–22) × (18–)18.3(–18.5) µm, emerging through stomata, globose, echinulate, wall 0.8–1.2 µm thick, cinnamon brown, germ pore inconspicuous (Fig. 2 C);

Telia: (0.2–)0.3(–0.4) × (0.1–)0.2(–0.3) cm, amphigenous, subepidermal, erumpent, sparse, separate, dark brown, powdery, found on mature leaves (Fig. 3 A). The distribution of telia occur closely or sparsely associated with each other, on leaves normally containing large aecial galls. (Fig. 4 A, B).

Teliospores: (36–)41(–46) × (17–)20(–24) µm, one-celled, clavate, obovoid to elliptical, pedicellate, light brown, brown to dark brown, pitted, reticulate, papillate, with one apical germ pore (Fig. 3 B–E), wall 0.8–1.0 µm thick and 1.2 µm at the apex, wall pits 0.6–1.3 µm in diam., papillae (0.6–)1(–1.3) high × (0.5–) 0.6 (–0.8) µm wide.

Notes:

Notably, the *Uromyces* species that are generally found on *Loranthaceae* is undoubtedly different from *U. hawksworthii*. *U. phtirusae* MAYOR on *P. pyrifolia* was evaluated by JACKSON (1927), who reported that some previously described pseudoteliospores were the true urediniospores having many excrescencies and four germ pores. In addition, he noted that the teliospores were verrucous and sometimes arranged linearly. In the key of the *Uromyces* species found on *Loranthaceae*, *U. phtirusae* shows longitudinally ridged urediniospores and minutely verrucose, reticulate, or sometimes striate short-pedicellate teliospores (CUMMINS 1939). HENNEN & al. (2005) followed the key of CUMMINS (1939), but ascribed (invalidly) the new name *U. neophtirusae* for *U. phtirusae*. *Uromyces ornatipes*, *U. euphlebius*, and *U. socius* are easily separated from *U. hawksworthii* because they all have longitudinally striate teliospores, unlike those found in the new species. The demicyclic species *U. circumscriptus* and *U. urbanianus* have smaller teliospores with inconspicuously reticulate surfaces. *Uromyces evastigatus* on *P. pyrifolia* in Colombia is known to produce long- to short-pedicellate teliospores with reticules measuring 1–1.5 µm and thick spore apices (6–9 µm) (CUMMINS 1939). Therefore, these three species are also clearly different from the specimens obtained from the Brazilian Cerrado, *U. hawksworthii*.

Tab. 1. Alignment of the clone sequences of the fungus during aecial (left) and telial (right) phases obtained from BLAST.

	Similarity (%)		Similarity (%)
Clone 1 x Clone 5	98.1	Clone 3 x Clone 5	99.7
Clone 1 x Clone 6	98.2	Clone 3 x Clone 6	99.9
Clone 2 x Clone 5	99.6	Clone 4 x Clone 5	99.1
Clone 2 x Clone 6	98.3	Clone 4 x Clone 6	98.5

Molecular identification

PCR amplification resulted in six clones from the genomic DNA obtained from the aeciospores and teliospores. The amplified and purified fragments of the six sequences obtained matched that of the *Uromyces hawksworthii* species (Accession number

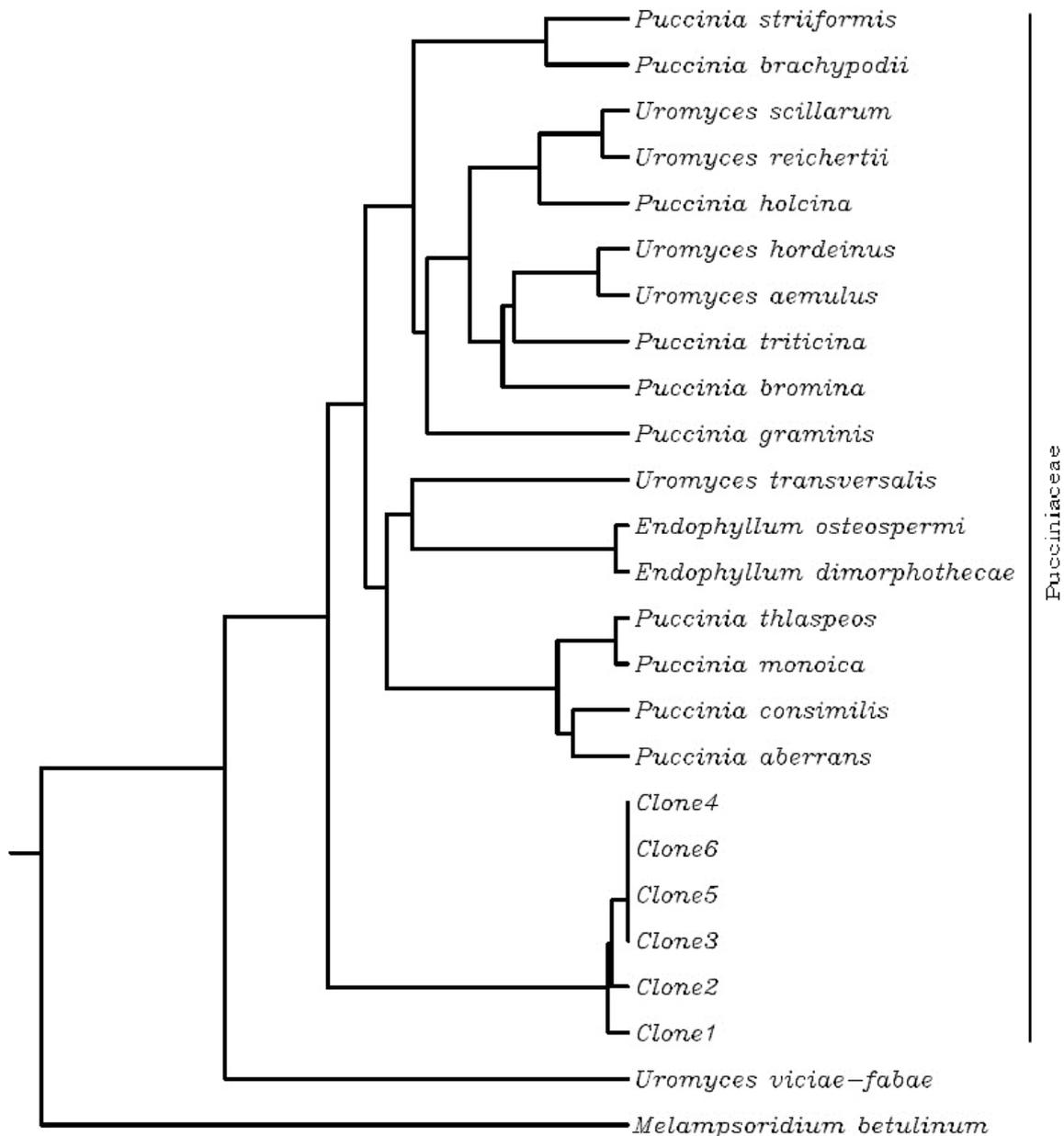


Fig. 5. Phylogenetic analysis of the clones 1–6 of the ITS sequence, showing the relationship between these clones and other rust fungi.

NR_132920, E-value = 0.0) with 100% similarity, according to a BLAST GenBank search. The sequences were aligned with each other, and more than 99% similarity was observed between both stages, reaching to a maximum of 99.9% (Tab. 1). Based on the analysis, it is known that the six clones belong to the same species. The results of DNA sequences show that all clones shared 99% similarity with the referred species in the databank, which belongs to the *Uromyces* genus. These results revealed that the spores from the aecial (Clones 1–4) and telial stages (Clones 5 and 6) had a high similarity and clustered within the rust family *Pucciniaceae*, containing the genus *Uromyces* (Fig. 5). Since the sequences presented high identity, alignment was performed with the sequences deposited in the DDBJ and a phylogenetic tree was constructed based on the ITS region.

Discussion

Morphology

Uromyces hawksworthii is a macrocyclic, autoecious rust fungus, whose aecial stage was described as *A. goyazense* on the same host *P. stelis* in several articles (SACCARDO 1897, SYDOW & SYDOW 1924, HENNEN & al. 2005, SOUZA & al. 2015). Other species of *Uromyces* found on *Loranthaceae* were already described based on the telial phase (CUMMINS 1939, HENNEN & al. 2005). In addition, the identity of the aecial/uredinial and telial stages in the life cycle of this fungus was confirmed by molecular analysis. Considering the morphological features, this species produces one-celled pedicellate teliospores with an apical germ pore, and shows a small portion of basidia when germinating. Other species, such as *U. bahiensis* and *U. circumscriptus* are morphologically similar in aecidiospore size. However, peridial cells are absent in the aecidia of *U. circumscriptus* (PERDOMO-SANCHEZ & PIEPENBRING 2014). *Uromyces hawksworthii* is characterized by reticulate pitted teliospores that are smaller than those of all other known species of *Uromyces* found on *Loranthaceae*.

Identification by DNA blasting

The molecular blasting of the rust fungus with GenBank data, matched that of *U. hawksworthii* previously deposited in GenBank and registered in Mycobank by SOUZA & al. (2015), found on *Loranthaceae* in the Brazilian cerrado. The current research confirms the results found by these authors and furthermore adds the description of the uredinia and urediniospores morphologically and molecularly.

DNA sequencing of *Uromyces* spp. has revealed the presence of distinct clades: the isolates causing rust on chickpea, fenugreek, and alfalfa; those found on field clover and pea plants; isolates causing rust on bean and cowpea; and those infecting faba beans (BARILLI & al. 2011). Therefore, it was concluded that the *Uromyces* species could be genetically grouped according to their host plant. STEADMAN & al. (2002) proposed that there is a wide adoption of differential series and nomenclature systems for the physiological races of *U. appendiculatus*, which contribute to the elaboration of an internationally standardized classification methodology based on host infection. Furthermore, amplified fragment length polymorphic (AFLP) DNA analysis indicated that Eurasian isolates of *Puccinia jaceae* from different hosts exhibited unique fingerprint patterns primarily based on host/pathogenicity preference (YOURMAN & LUSTER 2004). Molecular markers were used to unravel the genomic organization of crown rust resistance caused by *P. coronata* in a segregating *Lolium perenne* population (MUYLLE & al. 2005).

Among the rust fungi (*Pucciniomycetes*), *Puccinia* and *Uromyces* are the two largest genera, comprising 4000 and 600 species, respectively (CUMMINS & HIRATSUKA 2003). These genera are morphologically distinguished by the cell number of teliospores, number of germ pores per cell, teliospore germination, presence or absence of paraphyses in sorus, the persistence of pedicels, ornamentation and staining of spores, and the presence or absence of uredinia. However, *Puccinia* and *Uromyces* cannot be

distinguished by their spermogonia, aecia, uredinia, or spores produced in these sori (MAIER & al. 2007). There are species having one- and two-celled teliospores or up to three or four celled ones. Based on the characteristics of the life cycles of *Puccinia* and *Uromyces*, some taxonomists have indicated that phylogenetically, they could be a large group of genera (MAIER & al. 2007). This group is called the *Puccinia/Uromyces* complex (BURITICA & al. 2014; MAIER & al. 2003, 2007; WINGFIELD & al. 2004). Depending on the host biomes and the evolution of rust fungi, *Puccinia/Uromyces* can be separated by their hosts (VAN DER MERWE & al. 2007, MAIER & al. 2007). According to these authors, *Puccinia* s. l. and *Uromyces* s. l. are polyphyletic, but both *Uromyces* s. str. (type *U. appendiculatus*), and *Puccinia* s. str. (type *P. graminis*) have distinct clades. Phylogenetically, the *Uromyces* species are usually more spread, suggesting that they are older than the *Puccinia* species, and are separated into two major clades (VAN DER MERWE & al. 2007). The one-celled teliospores found among two-celled teliospores in the *Puccinia* species are mesospores. However, two-celled teliospores in *Uromyces* are rarer. Therefore, the molecular data support the hypothesis that the small number of teliospore cells in *Uromyces* represent a more advanced developmental stage (VAN DER MERWE & al. 2007). The molecular data show that there is a monophyletic group of *Uromyces* spp. on *Fabaceae*, whose species type is *U. appendiculatus*, since few *Puccinia* species are reported on *Fabaceae*. Other data have demonstrated a strong relationship between *Uromyces pisi* (heteroecious) with the alternate host belonging to *Euphorbiaceae* and *U. viciae-fabae* (autoecious). These two species are in a separate clade (VAN DER MERWE & al. 2007). VAN DER MERWE & al. (2007) noted a clear separation of different molecular characteristics within the clade, specifically the two groups of the *Uromyces* and *Puccinia* species, and indicated that the two genera are strictly distinguished. Considering this, a simple teliospore septum separates *Puccinia* and *Uromyces*. AIME & al. (2006) showed that *Puccinia* and *Uromyces* are polyphyletic.

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