

# A New Rust Disease on Wild Coffee (*Psychotria nervosa*) Caused by *Puccinia mysuruensis* sp. nov.

S. Mahadevakumar, Mycology and Phytopathology Laboratory, Department of Studies in Botany, University of Mysore, Manasagangotri, Mysuru, 570006, Karnataka, India; Les J. Szabo, United States Department of Agriculture–Agricultural Research Service, Cereal Disease Laboratory and Department of Plant Pathology, University of Minnesota, St. Paul, 55108; T. Eilam and Y. Anikster, Department of Botany and Institute for Cereal Crops Improvement, Tel Aviv University, Ramat Aviv, 69978, Israel; and G. R. Janardhana, Mycology and Phytopathology Laboratory, Department of Studies in Botany, University of Mysore, Mysuru, 570006, Karnataka, India

## Abstract

Mahadevakumar, S., Szabo, L. J., Eilam, T., Anikster, Y., and Janardhana, G. R. 2016. A new rust disease on wild coffee (*Psychotria nervosa*) caused by *Puccinia mysuruensis* sp. nov. *Plant Dis.* 100:1371-1378.

*Psychotria nervosa*, commonly called “wild coffee” (Rubiaceae), is an important ethno-medicinal plant in India. In 2010, a new rust disease of *P. nervosa* was observed in three regions of Mysore District, Karnataka (India), with disease incidence ranging from 58 to 63%. Typical symptoms of the rust disease on wild coffee were prominently visible during the early monsoon season (May to June), with chlorotic spots on the adaxial and black pustules (telia) on the abaxial leaf surface. Telia produced abundant teliospores, which were bicelled, pedicellate, and measured 33 to 45 by 19 to 30  $\mu\text{m}$ . The germination of teliospores produced a typical metabasidium bearing four basidiospores, each containing two haploid nuclei. Spore stages of the wild coffee rust pathogen

were studied using artificially inoculated healthy wild coffee plants with germinated teliospores. Only telia were observed on the inoculated plants, indicating that this rust fungus has an abbreviated microcyclic life cycle that includes only teliospores and basidiospores. Phylogenetic analysis based on internal transcribed spacer and partial large subunit (LSU) sequence data showed that the wild coffee rust pathogen is related to *Macruropycis fraxini*, *Puccinia bartholomaei*, *P. choridis*, and *P. sparganioidis*. The herbarium sample of *P. psychotriae* was examined and was shown to be different with respect to telium size and teliospore dimensions (24 to 32 by 13 to 18  $\mu\text{m}$ ). Therefore, the rust pathogen causing wild coffee rust is a new species, *P. mysuruensis* sp. nov.

Rust fungi (phylum Basidiomycota, subphylum Pucciniomycotina, Order Pucciniales) constitute one of the largest groups of basidiomycetes. More than 7,000 species (in over 100 genera) of rust fungi have been reported (Aime 2006; Aime et al. 2006; Cummins and Hiratsuka 2003; Hooker 1967). *Puccinia*, the largest genus, consists of over 4,000 species and is characterized by the production of pedicellate, bicelled teliospores. *Puccinia* spp. cause some of the most destructive diseases on crop plants (Cummins 1959; Hiratsuka 1955; Laundon and Waterston 1965; Thirumalachar and Mundkur 1949, 1950). Rust fungi have been divided into three categories depending upon the number of spore states in their life cycle: macrocyclic, demicyclic, and microcyclic (Cummins and Hiratsuka 2003). Typical macrocyclic rust fungi produce five spore states (teliospores, basidiospores, spermatia [pynciospores], aeciospores, and urediniospores), which occur on either a single host (autoecious) or two hosts (heteroecious). Absence of the uredinial spore state is classified as demicyclic and absence of both uredinial and aecial spores states as microcyclic.

*Psychotria nervosa* Sw. (Rubiaceae) is an evergreen, shade-tolerant shrub that is native to Florida and Central America; it is also commonly found in scrub jungles in India (Liogier 1997; Stevens et al. 2001). This shrub is commonly called wild coffee, because the fruit resembles the true coffee bean (Gilmen 2011). Different parts of this plant are used in ethno-medicinal preparations, especially by tribes of southern India and Bangladesh, for treatment of dysentery, colds, stomach ache, asthma, sores, boils, swollen feet, and fungal infection (Biswas et al. 2010; Porto et al. 2009).

During a survey (2010 to 2013), a new rust disease was observed on *P. nervosa* in three regions of the Mysore District of Karnataka State, India. The present study reports (i) description of the disease; (ii) characterization of the fungal pathogen, including spore morphology, germination of teliospores, inoculation studies, and phylogenetic study based on partial sequence of internal transcribed spacer (ITS) large subunit (LSU) ribosomal DNA (rDNA); (iii) description of the herbarium sample of *Puccinia psychotriae*; and (iv) description of a new rust pathogen species, *P. mysuruensis*.

## Materials and Methods

**Sample collection and disease assessment.** A rust disease on *Psychotria nervosa* was observed in three regions of Mysore District (Doddamaragowdana Hally: 12°17.145' N, 076°30.892' E; Mada Hally: 12°17.149' N, 076°30.911' E; and G. B. Sargur: 12°16.171' N, 076°29.368' E). Disease incidence was assessed during 2010, 2011, 2012 and 2013. In total, 891 wild coffee plants were evaluated in three different sites (Table 1). Disease incidence was estimated using total number of plants observed and number of plants with rust symptoms. In total, 10 leaves from each 10 plants ( $n = 100$  leaves) were collected and disease severity was assessed using an ordinal disease scale of 1 to 5 (Morin et al. 2012), where 1 indicates no visible symptoms; 2 indicates chlorotic, yellow spots on the adaxial surface, a protrusion on the abaxial surface; 3 indicates purplish, necrotic flecks, spots with underdeveloped telia; 4 indicates fully developed telia that covered less than 25% of the leaf surface area; and 5 indicates fully developed telia with a purplish halo that covered more than 25% of the leaf surface. A representative voucher specimen with well-developed disease symptoms was deposited in the Herbarium of the Department of Studies in Botany, University of Mysore (accession number: UOMMGB-201011).

**Micromorphological studies.** Fresh leaf samples with rust symptoms were examined using a Carl Zeiss Stereo Discovery microscope (Carl Zeiss Microscopy, LLC). Teliospores from fresh leaf samples were mounted in 50% glycerol and viewed with a bright-field fluorescent microscope (Olympus BX51), and digital images were obtained with a SPOT RT digital camera (Olympus Corporation). Dried and herbarium leaf samples containing rust fungal structures were rehydrated in

Corresponding author: G. R. Janardhana; E-mail: grjbelur@gmail.com

\*The e-Xtra logo stands for “electronic extra” and indicates that two supplementary tables are published online.

Accepted for publication 10 February 2016.

a moist chamber for 18 h, stained with lactophenol aniline blue (Remel; Lenexa), and mounted in 50% glycerol. For these samples, teliospores were examined using a Nikon Eclipse 90i microscope using NIS Elements AR software v 4.30 (Nikon Instruments Inc.). Teliospore dimensions (length, width, and cross-sectional area) were determined as described by Anikster et al. (1997, 2004). Staining of nuclei was done as described by Anikster et al. (2004).

The type collection of *Puccinia psychotriae* (3152) was obtained from the Herbarium Hamburgense, University of Hamburg for examination. This represents the only known collection of *P. psychotriae* (Hennen et al. 2005). Hennings (1905) first published a description of *P. psychotriae* and a partial English translation was included in

Hennen et al. (2005). In these publications, uredinia or telia size and images of the spores were not included. Therefore, examination and further description of the herbarium specimen was warranted. Teliospore and urediniospores dimensions were determined as described above.

**Induction of teliospore germination.** Teliospores were induced to germinate using six different conditions (Anikster 1986; Anikster et al. 2004). Collected teliospores from infected dried leaf material were floated on distilled water of a clean cavity slide, and incubated under the following conditions: (i) 4 to 8°C under a photoperiod of 12 h of darkness and 12 h of light up to 24 h; (ii) 12 to 16°C under continuous light up to 24 h; and (iii) 12 to 16°C under complete

**Table 1.** Disease incidence and severity of wild coffee rust recorded in Mysore District of Karnataka, India<sup>w</sup>

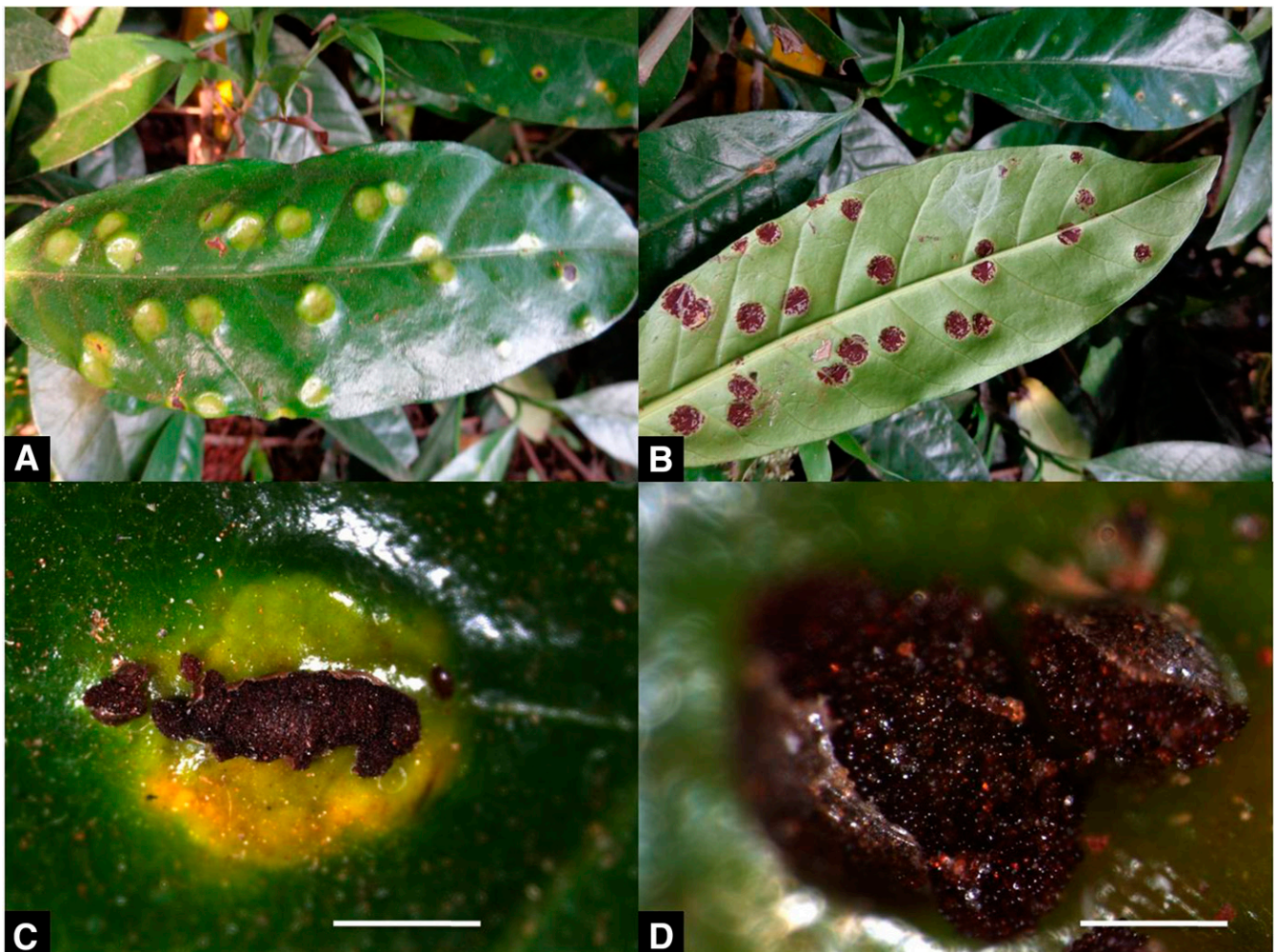
| Location <sup>x</sup> | 2010                 |          | 2011                 |          | 2012                 |          | 2013                 |          |
|-----------------------|----------------------|----------|----------------------|----------|----------------------|----------|----------------------|----------|
|                       | Incidence (%)        | Severity | Incidence (%)        | Severity | Incidence (%)        | Severity | Incidence (%)        | Severity |
| DMG                   | 66 (85) <sup>y</sup> | 4        | 57 (89) <sup>y</sup> | 3        | 51 (78) <sup>y</sup> | 5        | 63 (92) <sup>y</sup> | 4        |
|                       | ...                  | ...      | ...                  | ...      | 49 (65) <sup>z</sup> | ...      | 60 (68) <sup>z</sup> | 3        |
| Mada                  | 45 (40) <sup>y</sup> | 3        | 51 (45) <sup>y</sup> | 4        | 36 (42) <sup>y</sup> | 4        | 38 (42) <sup>y</sup> | 3        |
|                       | ...                  | ...      | ...                  | ...      | 58 (38) <sup>z</sup> | 4        | 48 (48) <sup>z</sup> | ...      |
| GB                    | ...                  | ...      | 47 (38) <sup>y</sup> | 2        | 60 (45) <sup>y</sup> | 3        | 43 (35) <sup>y</sup> | 3        |
|                       | ...                  | ...      | ...                  | ...      | 34 (41) <sup>z</sup> | 2        | ...                  | ...      |

<sup>w</sup> Disease severity was rated using a scale of 1 to 5. For incidence, values within parenthesis indicate the total number of plants observed.

<sup>x</sup> DMG = Doddamaragowdana Hally, Mada = Mada Hally, and GB = G.B. Sargur.

<sup>y</sup> Ratings were made between April and May.

<sup>z</sup> Ratings were made between September and November.



**Fig. 1.** Symptoms of the wild coffee rust pathogen (*Puccinia mysuruensis*) on *Psychotria nervosa*. **A**, Chlorotic patches on adaxial leaf surface. **B**, Telia on abaxial surface. **C**, Chlorotic patch with telia. **D**, Telium showing teliospores. C and D scale bar = 2 mm.

darkness up to 24 h. After the initial incubation period, teliospores were placed on 1.5% water agar (amended with chloramphenicol at 5 mg/liter) and incubated at room temperature ( $26 \pm 2^\circ\text{C}$ ) for 3 to 5 days and observed regularly for the germination of teliospores: (iv) teliospores from infected tissue were floated on water and incubated at  $12$  to  $16^\circ\text{C}$  under a photoperiod of 12 h of light and 12 h of darkness up to 24 h; (v) leaf segments with telia were incubated in a moist blot chamber at  $12$  to  $16^\circ\text{C}$  under a photoperiod of 12 h of light and 12 h of darkness for up to 24 h; and (vi) leaf segments with telia were cut out and incubated in a moist chamber at  $12$  to  $16^\circ\text{C}$  for 24 h under continuous darkness. Teliospores were then scraped off and placed on a cavity slide with water at the same temperature. At regular intervals, teliospore samples were removed and examined for germination. These six treatments were repeated using fresh leaf samples containing telia. Observations were made at 1, 3, 6, 12, 18, 24, 36, and 48 h, and at the onset of teliospore germination and basidium development was recorded. The experiments were repeated thrice and, in each experiment, 100 teliospores ( $n = 100$ ) were evaluated for germination.

**Inoculation studies under field conditions.** Experiments in the field were performed at three different sites (Doddamaragowdana Hally, Mada Hally, and G.B. Sargur) with wild *Psychotria nervosa* plants (2 years old) to determine life cycle and pustule development. Experiments were conducted after a period of continuous rain for 3 to 4 days with 85% relative humidity during two seasons (October 2012 and May 2013). Disease-free plants were inoculated with a teliospore suspension ( $1 \times 10^5$  teliospores  $\text{ml}^{-1}$ ) by spraying the foliage of 10 healthy plants until the leaves were fully wet and the spore suspension began to run off. The treated foliage was then covered with a polythene bag for up to 5 days. As a control, healthy leaves were

sprayed with sterile distilled water. Development of disease symptoms was recorded after 30 days postinoculation. Photographs were taken from initial symptoms through complete development of telia.

**Polymerase chain reaction amplification, sequencing ITS-LSU of rDNA, and phylogenetic analysis.** Total DNA was extracted from infected leaf material as described by Anikster et al. (2004). Briefly, dried infected leaf sample was pulverized by grinding in tubes with 1-mm glass beads (Lysing Matrix C; Bio 101) and 25 mg of diatomaceous earth (Sigma-Aldrich) using a Savant FastPrep shaker (FP120). DNA was extracted using an OmniPrep DNA extraction kit (G-Biosciences), as described by the manufacturer. ITS1F and RUST1 primers were used for polymerase chain reaction (PCR) amplification and the resulting products were purified, cloned, and sequenced (Anikster et al. 2004). At least three clones per sample were sequenced completely across both strands. DNA sequence analysis was performed using the software package Geneious (v 8.1.8; Biomatters). Blast search of the GenBank nonredundant sequence database (conducted 22 December 2015) was used to identify sequences of rust fungi that were related for phylogenetic analysis (Supplementary Table S1). DNA sequences were aligned using the module MAFFT v 7.017 (Katoh et al. 2002), with default settings and ends trimmed so that all sequence alignments were of equal length. Phylogenetic analysis was performed using PhyML module (Guindon and Gascuel 2003) with 1,000 bootstrap replicates.

For the *Puccinia psychotriae* herbarium sample, combinations of ITS1F, ITS1rustF10d (Barnes and Szabo 2007; Gardes and Bruns 1993), and Rust2inv (Aime 2006) as forward primers and RUST1, ITS2, ITS4, and ITS4B (Kroop et al. 1995; White et al. 1990) as reverse primers were used, as described by Anikster et al. (2004). PCR reactions contained 0.005% casein,  $1 \times$  PCR PlatinumTaq PCR mix,

**Table 2.** Comparison of spore dimensions between *Puccinia mysuruensis* sp. nov. and *P. psychotriae*<sup>x</sup>

| Sample <sup>y</sup>   | N <sup>z</sup> | Length ( $\mu\text{m}$ ) |           | Width ( $\mu\text{m}$ ) |           | Area ( $\mu\text{m}^2$ ) |         |
|-----------------------|----------------|--------------------------|-----------|-------------------------|-----------|--------------------------|---------|
|                       |                | Mean $\pm$ SD            | Range     | Mean $\pm$ SD           | Range     | Mean $\pm$ SD            | Range   |
| <i>P. mysuruensis</i> |                |                          |           |                         |           |                          |         |
| Teliospores           |                |                          |           |                         |           |                          |         |
| Fresh                 | 75             | $38.1 \pm 2.9$           | 32.7–44.5 | $25.0 \pm 2.2$          | 19.1–29.8 | nd                       | nd      |
| Dried                 | 51             | $37.7 \pm 2.5$           | 33.2–43.8 | $24.6 \pm 1.2$          | 22.0–26.8 | $754 \pm 51$             | 648–874 |
| Combined              | 126            | $37.9 \pm 2.7$           | 32.7–44.5 | $24.8 \pm 1.8$          | 19.1–29.8 | nd                       | nd      |
| <i>P. psychotriae</i> |                |                          |           |                         |           |                          |         |
| Teliospores           |                |                          |           |                         |           |                          |         |
| Type                  | 23             | $27.9 \pm 2.3$           | 23.7–32.1 | $15.2 \pm 1.1$          | 12.6–17.8 | nd                       | nd      |
| Hennings              | ...            | nd                       | 30–35     | nd                      | 13–16     | nd                       | nd      |
| Urediniospores        |                |                          |           |                         |           |                          |         |
| Type                  | 40             | $20.0 \pm 1.9$           | 16.6–23.3 | $15.7 \pm 1.1$          | 13.6–18.0 | $239 \pm 19$             | 202–272 |
| Hennings              | ...            | nd                       | 15–22     | nd                      | 10–16     | nd                       | nd      |

<sup>x</sup> SD = standard deviation and nd = no data.

<sup>y</sup> Fresh = fresh spores, Dried = dried sample rehydrated and stained with lactophenol-aniline blue, Combined = combined data from fresh and dried samples, Type = herbarium sample rehydrated and stained with lactophenol-aniline blue (Herb. Hamburg Number 3152), and Hennings = data from Hennings(1905).

<sup>z</sup> Number of spores.

**Table 3.** Germination (percent  $\pm$  standard error) of teliospores of *Puccinia mysuruensis* sp. nov.<sup>w</sup>

| Trt <sup>x</sup> | T ( $^\circ\text{C}$ ) <sup>y</sup> | Photoperiod <sup>z</sup> | Fresh leaves       |       |       | Dried leaves       |       |       |
|------------------|-------------------------------------|--------------------------|--------------------|-------|-------|--------------------|-------|-------|
|                  |                                     |                          | Germination (%)    | Metab | Basid | Germination (%)    | Metab | Basid |
| 1                | 4–8                                 | 12/12                    | ...                | –     | –     | ...                | –     | –     |
| 2                | 12–16                               | 24/0                     | ...                | –     | –     | ...                | –     | –     |
| 3                | 12–16                               | 0/24                     | $27.66 \pm 4.33$ c | +     | +     | $7.66 \pm 1.45$ b  | +     | –     |
| 4                | 12–16                               | 12/12                    | $24.00 \pm 3.05$ c | +     | +     | $12.00 \pm 1.52$ b | +     | +     |
| 5                | 12–16                               | 12/12                    | $56.33 \pm 4.48$ b | +     | +     | $13.33 \pm 1.66$ b | +     | +     |
| 6                | 12–16                               | 0/24                     | $79.00 \pm 4.58$ a | +     | +     | $32.66 \pm 1.45$ a | +     | +     |

<sup>w</sup> For metabasidium (Metab) and basidiospore (Basid), – indicates absent and + indicates present. Values are the mean of three replications. Mean followed by the same letters within the column are not significantly different according to Tukey's honestly significant difference.

<sup>x</sup> For treatments (Trt) 1 through 4, teliospores were floated on water for 24 h, then transferred to water agar (1.5%) plates. For treatments 5 and 6, infected leaf segments with telia incubated in a moist chamber for 24 h; then, were teliospores removed and placed in cavity slide containing water.

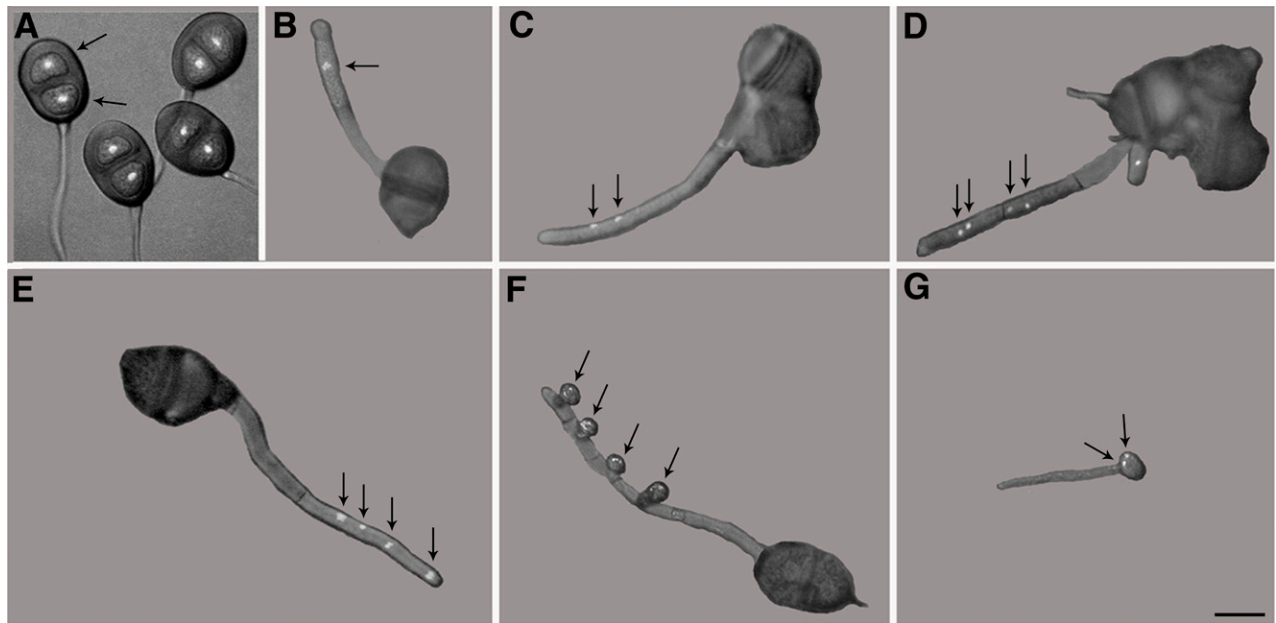
<sup>y</sup> Temperature.

<sup>z</sup> Photoperiod (light/darkness).

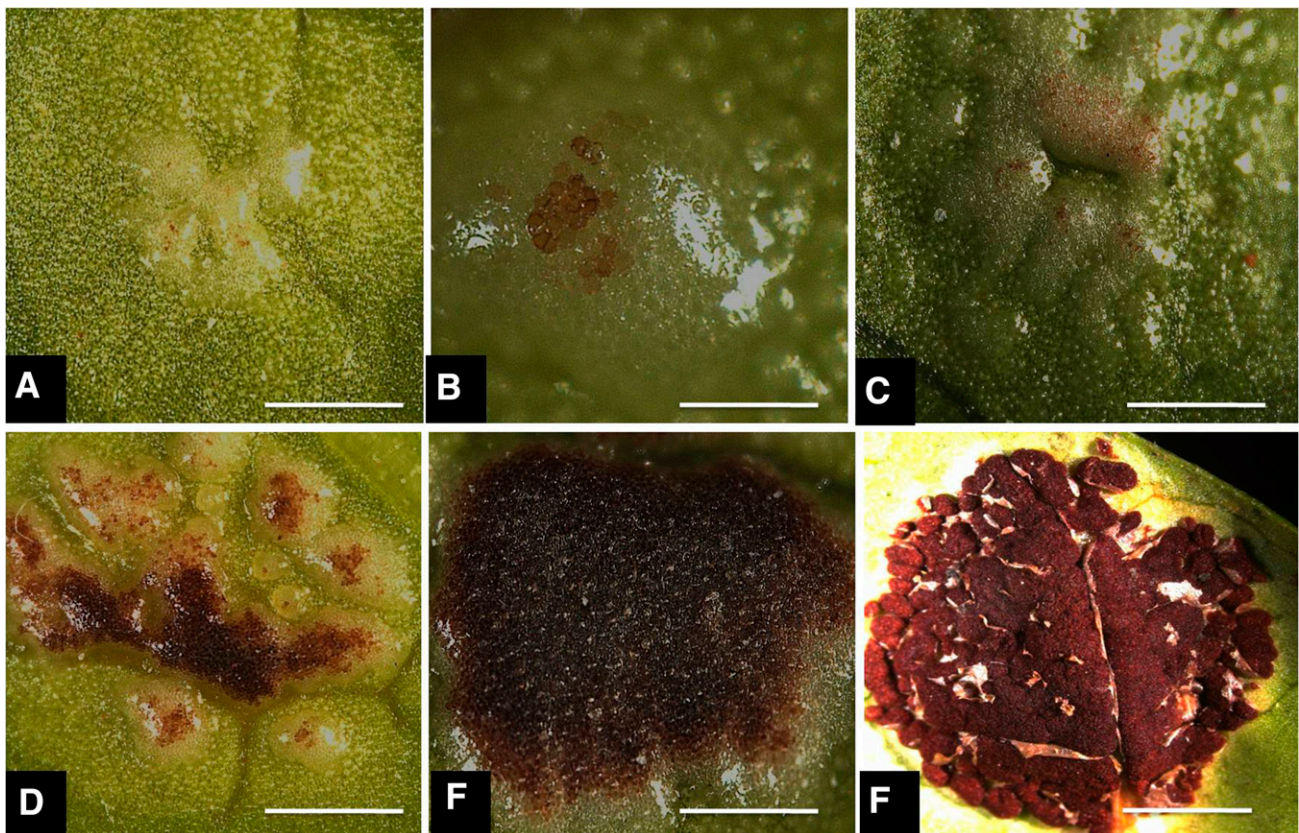
1.5 mM MgCl<sub>2</sub>, 0.2 mM dNTP each, 0.2 μM forward primer, 0.2 μM reverse primer, and 2 U of PlatinumTaq (Invitrogen). The PCR conditions were 94°C for 2 min; followed by 31 cycles of 94°C for 30 s, 44°C for 30 s, and 72°C for 2 min; and 72°C for 10 min with a hold at 4°C in a PCR Thermal Cycler (Model PTC-200, MJ Research). The

annealing temperature was varied depending on the primer combinations used.

**Statistical analysis.** Statistical analysis of teliospore dimensions was performed using the program Wizard v1.7.19 ([www.wizardmac.com](http://www.wizardmac.com)) with Mann-Whitney test to determine whether the median values were



**Fig. 2.** Nuclear state of teliospore, metabasidium, and basidiospore. **A**, Teliospore with diploid nuclei. **B**, Migration of the diploid nucleus into the metabasidium. **C**, Two nuclei in the metabasidium after the first meiotic division. **D**, Four haploid nuclei in the metabasidium as the result of the second meiotic division (a different metabasidium containing migrated diploid nucleus is seen). **E**, Post-meiosis division of the metabasidium to four cells (+an empty basal cell) each contains one haploid nucleus. **F**, Mature metabasidium with four binucleate basidiospores (as a result of a third mitotic division). **G**, Germinating basidiospore. Scale bar = 20 μm.



**Fig. 3.** Telium development of the wild coffee rust pathogen (*Puccinia mysuruensis*) on the abaxial leaf surface of *Psychotria nervosa*. **A**, Formation of white patches. **B** and **C**, Development of protrusions with dark spots. **D** and **E**, Expansion of protrusions and coalescence of dark spots. **F**, Mature telium. **A** to **E**: scale bar = 2 mm; **F**: scale bar = 4 mm.

different between sets. Data on teliospore germination were subjected to analysis of variance using SPSS Inc. 16.0. Significant effects of treatments were determined by the magnitude of the  $F$  value ( $P \leq 0.05$ ) and means were separated by Tukey's honestly significant difference test.

## Results

**Sample collection and disease assessment.** The development of rust disease on *Psychotria nervosa* was observed during the rainy seasons (April to May and September to November) under field conditions of high relative humidity (>85%) lasting for a minimum of 5 days. Initial symptoms consisted of chlorotic spots (2 to 5 mm) on the adaxial surface of the leaf, followed by circular black pustules on the abaxial leaf surface (Fig. 1). Mature pustules ranged in diameter from 2 to 12 mm. In some instances, a telium erupted through the chlorotic spots on the adaxial leaf surface. Occasionally, telia were observed on stems. The spermogonia (pycnia), aecia, and uredinia stages of the wild coffee rust fungus were not observed on *P. nervosa* during the course of this study.

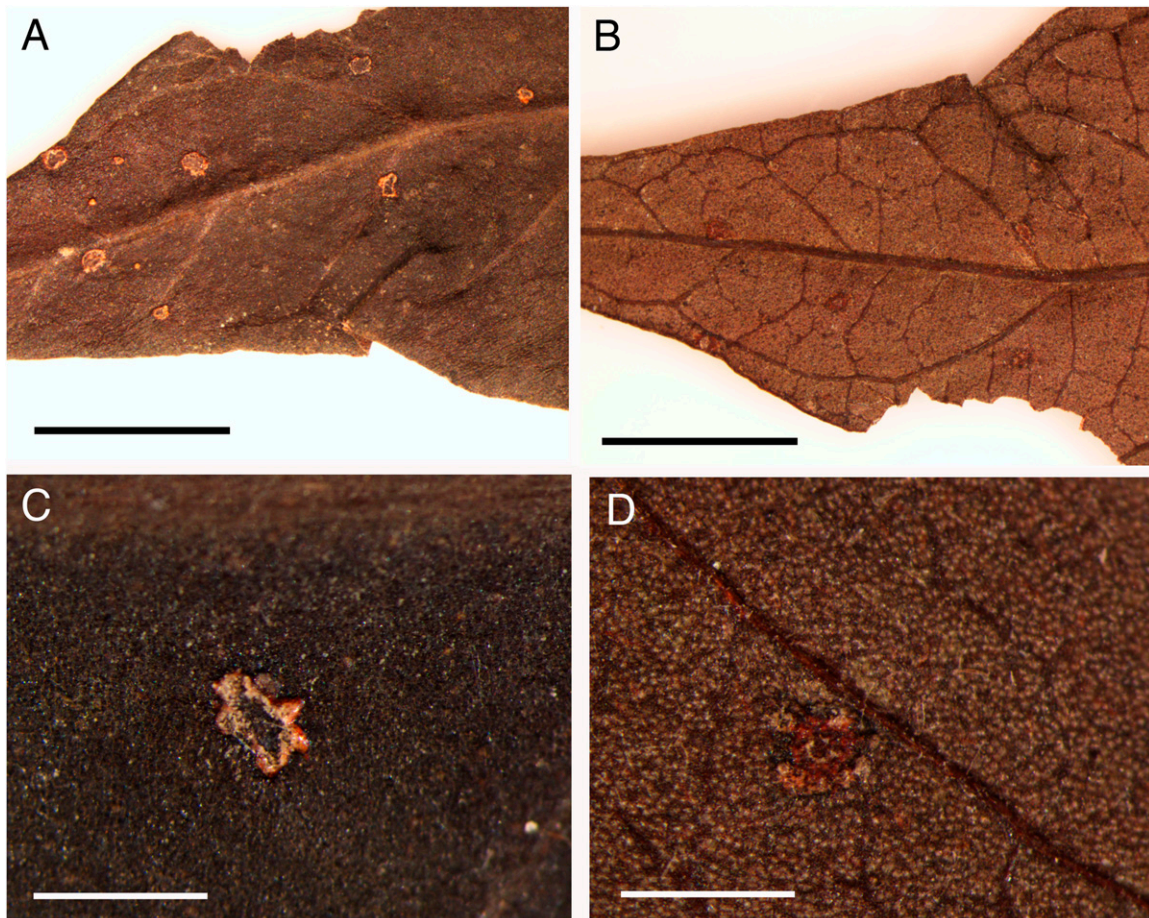
The disease incidence and severity of wild coffee rust was evaluated during the rainy seasons in 2010 to 2013 at three different locations in Mysore District of Karnataka, India. The disease incidence ranged from 34 to 66% and severity from 2 to 5 (Table 1).

**Micromorphological studies.** Teliospores were two-celled, broadly oblong to clavate, rounded above, attenuate below, slightly constricted at the septum, and smooth. Teliospore dimensions (length, width, and cross-sectional area) of teliospores from fresh and dried samples were measured, as well as cell wall thickness. No significant differences were found between the measurements from fresh or dried teliospore samples ( $P = 0.358$ ). Therefore, the data sets for length and width were combined (Table 2). Pedicel were hyaline, persistent, and with a length up to 182  $\mu\text{m}$  ( $n = 50$ ).

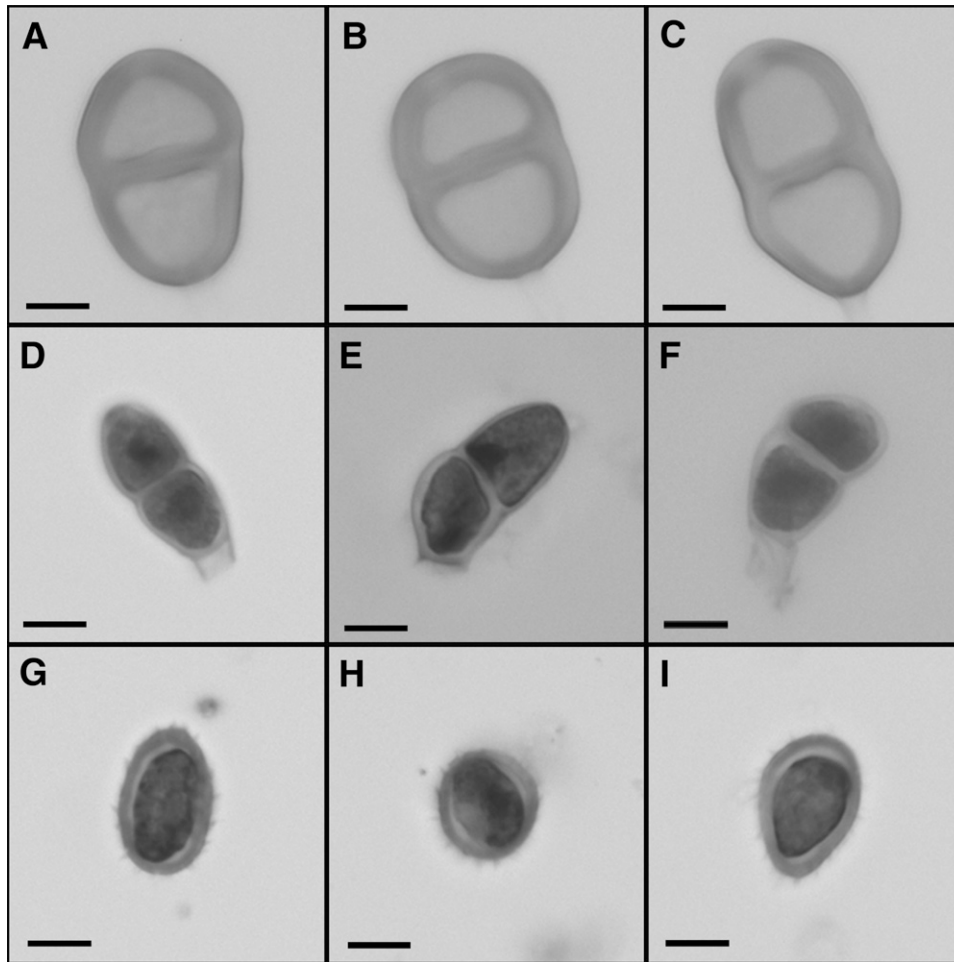
**Teliospore germination.** The greatest germination of teliospores was observed with fresh, infected leaf material with telia incubated in a moist chamber at 12 to 16°C for 24 h in the dark, followed by incubating just teliospores on a cavity slide with sterile water, with a mean germination rate of 70% with complete metabasidium and basidiospore development (Table 3). Within 18 h of transfer to the cavity slide, teliospores with fully developed metabasidia and basidiospores were observed. Metabasidia typically contained four cells, each producing a sterigma and basidiospore. Germinated basidiospores were often observed. In some cases, both cells of a teliospore germinated (data not shown). Nuclear staining showed the typical progression of the teliospore germination from diploid nucleus (Fig. 2A), through nuclear migration into the metabasidium (Fig. 2B), followed by meiotic division (Fig. 2C and D) and formation of four haploid cells (Fig. 2E). During basidiospore formation, nuclear migration followed by mitotic division occurred (Fig. 2F), which resulted in mature basidiospores with two haploid nuclei (Fig. 2G). Mature basidiospores were hyaline, ovate, elliptical, and smooth (9.93 to 18.21 by 6.3 to 7.93  $\mu\text{m}$ ).

**Inoculation studies conducted under field conditions.** When healthy leaves of *P. nervosa* were inoculated with fresh teliospores suspended in water, the first symptom was observed 4 to 5 days post-inoculation (dpi), consisting of small chlorotic spots on the adaxial surface of the leaf. Fully developed telia were observed on the abaxial leaf surface 11 to 12 dpi. Inoculation studies under field conditions produced disease incidence of 50, 20, and 30% at Doddamaragowdana Hally, Mada Hally, and G.B. Sargur, respectively. No disease was observed on mock-inoculated control plants.

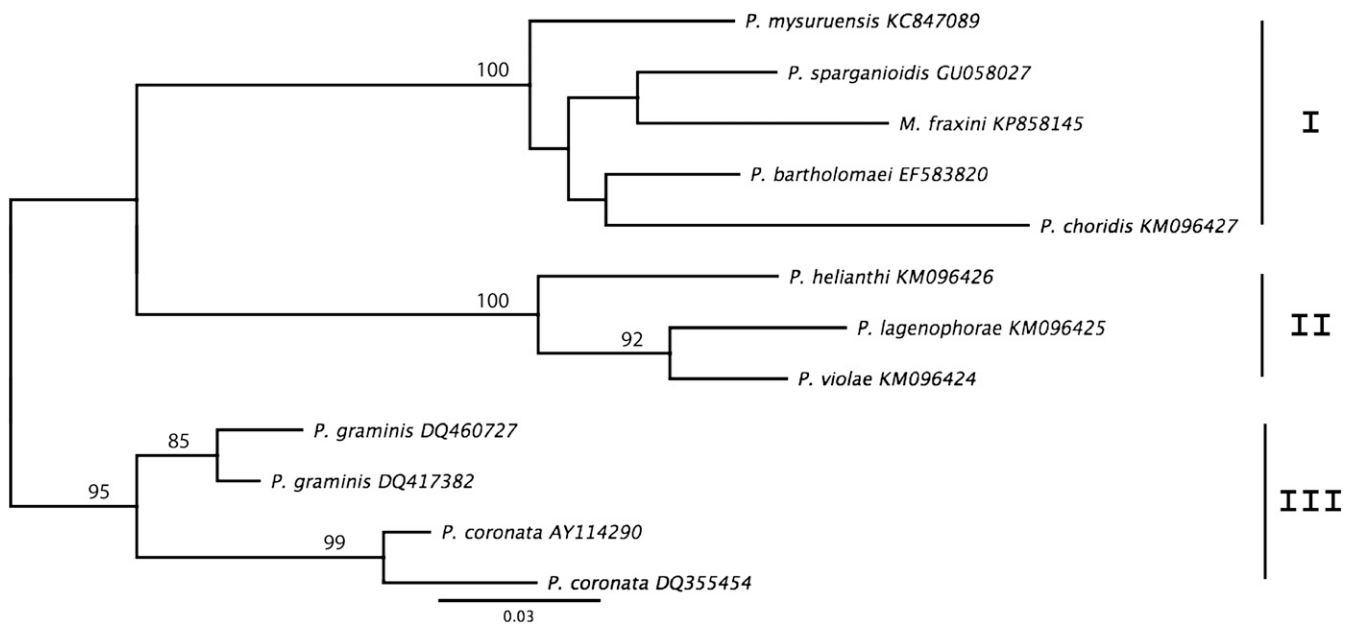
Telium development on the abaxial leaf surface progressed through four distinct phases. Initially, small white patches (<0.5 mm) were observed (Fig. 3A). In the next phase, protrusions developed and, as these expanded, dark spots appeared (Fig. 3B and C). As individual protrusions further expanded, these coalesced and turned brown or black



**Fig. 4.** Uredinia and telia of *Puccinia psychotriae* herbarium specimen. **A** and **C**, Uredinia on adaxial leaf surface. **B** and **D**, Telia on abaxial leaf surface. **A** and **B**: scale bar = 5 mm and **C** and **D**: scale bar = 1 mm.



**Fig. 5.** Morphology of teliospores and urediniospores of *Puccinia mysuruensis* and *Psychotria nervosa*. **A to C**, *Puccinia mysuruensis* teliospores; **D to F**, *P. psychotriae* teliospores; **G to I**, *P. psychotriae* urediniospores. Scale bar = 10  $\mu$ m.



**Fig. 6.** Phylogenetic tree of *Puccinia mysuruensis* sp. nov. and related rust fungi based on maximum-likelihood analysis of nuclear ribosomal DNA internal transcribed spacer 2 and partial large subunit sequences. GenBank accession numbers for each of the rust fungi are given. Bootstrap values for 1,000 replicates are shown (>75%). Branch lengths are proportional to genetic distance as measured by the number of substitutions per site. Clades with significant support are indicated.

from the center to the periphery (Fig. 3D and E). In the final phase, the mature teliospores erupted through the lower epidermal layer of the leaf (Fig. 3F). No paraphyses were observed in the telium.

**Analysis of *Puccinia psychotriae* herbarium specimen.** Uredinia on the adaxial side of the leaves were round to oblong and ranged in size from 0.3 by 0.3 mm to 1.0 by 0.7 mm (Fig. 4A and C). Telia on the abaxial side of the leaves were round to oblong with irregular borders and ranged in size from 0.3 by 0.3 mm to 0.9 by 0.7 mm (Fig. 4B and D). Very few teliospores were observed, sometimes mixed with urediniospores, whereas urediniospores were abundant. In general, the spores recovered from the herbarium sample were very similar to those described by Hennings (1905) but the teliospores were slightly shorter in length (Table 2).

Comparison of the pustule and spore morphology between the wild coffee rust fungus and *Puccinia psychotriae* indicated that these are different species. The telia of the wild coffee rust pathogen were larger (2 to 12 mm) than those from *P. psychotriae* (0.3 to 0.9 mm). In addition, the dimensions (length and width) of the teliospores of the wild coffee rust fungus were significantly larger than those of *P. psychotriae* (length,  $P < 0.001$  and width,  $P < 0.001$ ; Fig. 5).

**Analysis ITS rDNA.** The complete ITS region, including the 5.8S and approximately 550 bp of the 5' end of the LSU of the nuclear rDNA repeat, was cloned and sequenced from *P. mysuruensis* (GenBank accession number KC847089). All attempts to clone and sequence the complete ITS region or portions of it from the herbarium sample of *P. psychotriae* were unsuccessful.

Blast search of the GenBank nonredundant sequence database was unable to identify a match to the *P. mysuruensis* sequence; however, several highly related sequences were identified and used for analysis. Pairwise distance analysis based on percent identity of aligned sequences indicated that *P. mysuruensis* was most closely related to *P. sparganioidis* (ITS2, 87.54%) followed by *P. bartholomaei* (ITS2, 85.88%), *Macruropyxis fraxini* (ITS2, 85.59%), and *P. choridis* (ITS2, 83.78%) (Supplementary Table S2). Maximum-likelihood analysis showed that *P. mysuruensis* clustered with four other rust fungi (*M. fraxini*, KP858145; *P. bartholomaei*, EF583820; *P. choridis*, KM096427; and *P. sparganioidis*, GU058027), which formed a well-supported clade (Fig. 6). The nearest sister clade contained three additional rust species (*P. helianthi*, KM096427; *P. lagenophorae*, KM096425; and *P. violae*, KM096424), followed by a third clade containing representative members of the *P. graminis* (DQ460727 and DQ417382) and *P. coronata* (AY114290 and DQ355454) species complexes. These data are consistent with the morphological data supporting the idea that *P. mysuruensis* represents a new rust species.

**Taxonomy.** *Puccinia mysuruensis*, Mahadevakumar, Szabo & Janardhana, sp. nov. MycoBank MB812884.

**Etymology.** In recognition of the geographical location from where the wild coffee rust pathogen was first observed and described.

Telia scattered mostly on abaxial side of leaf, a few associated with chlorotic spots on adaxial side, dark chestnut brown, rounded and 3 to 8 mm diameter. Teliospores 33 to 44.5 by 19 to 29.8  $\mu\text{m}$  (39.7 by 24.8), broadly oblong to clavate, rounded above, attenuate below, slightly constricted at septum, smooth, spore wall 4 to 6  $\mu\text{m}$  thick apical and 2 to 3.5  $\mu\text{m}$  thick basal; pedicel hyaline and persistent, up to 180  $\mu\text{m}$  long. Spermogonia, aecia, and uredinia are lacking.

**Type on** *Psychotria nervosa*. Mysore District, India (UOMMGB-201011; BPI 893210).

## Discussion

A new rust disease was observed on wild coffee (*Psychotria nervosa*) in the scrub jungle forests of Mysore district, India during the rainy seasons from 2010 to 2013. This disease produced characteristic chlorotic spots on the upper leaf surface and typical brown to black patches (telia) on the lower leaf surface. The infected plants showed reduced growth, wilting of affected leaves during dry conditions, and loss of glossy appearance. Based on morphological and molecular data, the causal agent of wild coffee rust is described as a new species, *Puccinia mysuruensis*.

Based on inoculation and microscopic examination of germinating teliospores, *P. mysuruensis* was determined to have a microcyclic life

cycle consisting of only two spore stages (teliospores and basidiospores). Upon germination, teliospores produced a typical metabasidium containing four haploid cells, which produced four basidiospores. Microcyclic life cycle is reported in several genera of rust fungi, including *Coleosporium pinicola* (Smith 1970), *P. malvacearum* (Allen 1933; Blackman and Fraser 1906), *Ravenelia pringlei* (Petersen 1974), and *Tranzschelia thalictri* (Scholler et al. 2014). *P. malvacearum*, similar to *P. mysuruensis*, produces only telia and basidia, with four basidiospores. Studies by Allen (1935) showed that *P. malvacearum* has haploid hyphae within the leaf that fuse to form diploid hyphae and that these hyphae were from different basidiospore infections. Thus, *P. malvacearum* is heterothallic. Other rust fungi that exhibit a short life cycle lacking pycnia and aecia often form metabasidium, producing two basidiospores initially with two nuclei per cell, and are homothallic (Anikster and Wahl 1985; Anikster et al. 1977, 1980, 2004). Because *P. mysuruensis* forms a metabasidium with four cells and produces four basidiospores, it is assumed that it is also heterothallic; however, this needs further confirmation.

Three *Puccinia* spp. have been described that are pathogens of *Psychotria* spp. (Farr and Rossman 2015; Hennen et al. 2005), each of which is morphologically distinct from *P. mysuruensis*. *P. fallax* Arthur and *P. psychotriae* both produce uredinia and telia and the teliospore dimensions are smaller (*P. fallax*, 26 to 31 by 13 to 16  $\mu\text{m}$  and *P. psychotriae*, 30 to 35 by 13 to 16  $\mu\text{m}$ ). The third species, *P. palicoureae*, is very similar to *P. fallax* (Hennen et al. 2005). Currently, it is unclear what the evolutionary relationships of these three species are with *P. mysuruensis*. Phylogenetic analysis of DNA sequence data would help resolve this; however, in the case of *P. psychotriae*, the only known sample is the type herbarium specimen collected in 1901 and attempts to generate DNA sequence data were unsuccessful. At present, DNA sequence data are not available for either *P. fallax* or *P. palicoureae*. Clearly, additional work needs to be done to develop DNA sequence data for an expanded phylogenetic analysis.

To date, no rust disease has been recorded on *Psychotria nervosa*; thus, this is the first report of rust on wild coffee caused by a new rust species, *Puccinia mysuruensis*. Additional surveys are needed to determine the extent of this disease spread throughout Southeast Asia where *Psychotria nervosa* generally grows as wild plant. In the southern United States, *P. nervosa* is widely grown as an ornamental and the spread of the *Puccinia mysuruensis* could have negative effects on its use.

## Acknowledgments

We thank C. Manoharachary, Professor Emeritus, Osmania University, Hyderabad, India and K. R. Sridhar, UGC-BSR Faculty, Mangalore University, Mangalore, India for their help and critical comments; and K. P. Nguyen and A. Nemitz, United States Department of Agriculture–Agricultural Research Service Cereal Disease Laboratory, for technical assistance. S. Mahadevakumar (IF110422) acknowledges the Department of Science and Technology, New Delhi, India, for providing an Inspire Fellowship.

## Literature Cited

- Aime, M. C. 2006. Towards resolving family-level relationships in rust fungi (Uredinales). *Mycoscience* 47:112-122.
- Aime, M. C., Matheny, P. B., Henk, D. A., Frieders, E. M., Nilsson, R. H., Piepenbring, M., McLaughlin, D. J., Szabo, L. J., Begerow, D., Sampaio, J. P., Bauer, R., Weib, M., Oberwinkler, F., and Hibbett, D. 2006. An overview of the higher level classification of Pucciniomycotina based on combined analyses of nuclear large and small subunit rDNA sequences. *Mycologia* 98:896-905.
- Allen, R. F. 1933. A cytological study of the teliospores, promycelia and sporidia in *Puccinia malvacearum*. *Phytopathology* 23:572-586.
- Allen, R. F. 1935. A cytological study of *Puccinia malvacearum* from the sporidium to the teliospore. *J. Agric. Res.* 51:801-818.
- Anikster, Y. 1986. Teliospore germination in some rust fungi. *Phytopathology* 76:1026-1030.
- Anikster, Y., Bushnell, W. R., Eilam, T., Manisterski, J., and Roelfs, A. P. 1997. *Puccinia recondita* causing leaf rust on cultivated wheat, wild wheat and rye. *Can. J. Bot.* 75:2082-2096.
- Anikster, Y., Mosemann, J. G., and Wahl, I. 1977. *Uromyces viennot-bourginii*, Its Life Cycle, Pathogenicity and Cytology. Pages 9-17 in: *Travaux-Dedies-a-Georges-Viennot-Bourgin*, Societe Francaise de Phytopathologie, Paris.

- Anikster, Y., Moseman, J. G., and Wahl, I. 1980. Development of basidia and basidiospores in *Uromyces* species on wild barley and Liliaceae in Israel. *Trans. Br. Mycol. Soc.* 75:377-382.
- Anikster, Y., Szabo, L. J., Eilam, T., Manisterski, J., Koike, S. T., and Bushnell, W. R. 2004. Morphology, life cycle biology and DNA sequence analysis of rust fungi on garlic and chives from California. *Phytopathology* 94: 569-577.
- Anikster, Y., and Wahl, I. 1985. Basidiospore formation and self fertility in *Puccinia mesnieriana*. *Trans. Br. Mycol. Soc.* 84:164-167.
- Barnes, C. W., and Szabo, L. J. 2007. Detection and identification of four common rust pathogens of cereals and grasses using real-time polymerase chain reaction. *Phytopathology* 97:717-727.
- Biswas, A., Bari, M. A., Roy, M., and Bhandra, S. K. 2010. Inherited folk pharmaceutical knowledge of tribal people in Chittagong Hill tracts, Bangladesh. *Ind. J. Trad. Knowl.* 9:77-89.
- Blackman, V. H., and Fraser, H. C. I. 1906. Studies on the sexuality of the Uredineae. *Ann. Bot. (Lond.)* 20:35-48.
- Cummins, G. B. 1959. *Illustrated Genera of Rust Fungi*. Burgess, Minneapolis, MN.
- Cummins, G. B., and Hiratsuka, Y. 2003. *Illustrated Genera of Rust Fungi*, 3rd ed. American Phytopathological Society, St. Paul, MN.
- Farr, D. F., and Rossman, A. Y. 2015. *Fungal Databases*. Online publication. Systematic Mycology and Microbiology Laboratory, United States Department of Agriculture—Agricultural Research Service. <http://nt.ars-grin.gov/fungaldatabases/>
- Gardes, M., and Bruns, T. D. 1993. ITS primers with enhanced specificity for basidiomycetes: Application to the identification of mycorrhizae and rusts. *Mol. Ecol.* 2:113-118.
- Gilmen, E. F. 2011. *Psychotria nervosa* Wild Coffee, FPS 494. Online publication. University of Florida IFAS Extension. <https://edis.ifas.ufl.edu/fp494>
- Guindon, S., and Gascuel, O. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Syst. Biol.* 52:696-704.
- Hennen, J. F., Figueiredo, M. B., Caralho, A. A., Jr., and Hennen, P. G. 2005. Catalogue of the species of plant rust fungi (Uredinales) of Brazil. Online publication.
- Hennings, P. 1905. Fungi amazonici—IV. a cl. Ernesto Ulecollecti. *Hedwigia* 44: 57-71.
- Hiratsuka, N. 1955. *Uredinological Studies*. Kasai, Tokyo.
- Hooker, A. L. 1967. The genetics and expression of resistance in plants to rusts of the genus *Puccinia*. *Annu. Rev. Phytopathol.* 5:163-178.
- Katoh, K., Misawa, K., Kuma, K., and Miyata, T. 2002. MAFFT: A novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Res.* 30:3059-3066.
- Kroop, B. R., Albee, S., Flint, K. M., Zambino, P., Szabo, L. J., and Thompson, S. V. 1995. Early detection of systemic rust infections of Dyers Woad (*Isatis tinctori* L.) using polymerase chain reaction. *Weed Sci.* 43:467-472.
- Laundon, G. F., and Waterston, J. M. 1965. *Puccinia allii*. CMI Descriptions of Pathogenic Fungi and Bacteria. No. 52. Commonwealth Mycol. Inst., Kew, Surrey, UK.
- Lioigier, H. A. 1997. *Descriptive Flora of Puerto Rico and Adjacent Islands*, 5th Volume. Editorial de la Universidad de Puerto Rico Press, San Juan, PR.
- Morin, L., Aveyard, R., Lidbetter, J. R., and Wilson, G. P. 2012. Investigating the host range of the rust fungus *Puccinia psidii* sensu lato across the tribes of the family Myrtaceae present in Australia. *PLoS One* 7:e35434 .
- Petersen, R. H. 1974. The rust fungus life cycle. *Bot. Rev.* 40:453-513.
- Porto, D. D., Henriques, A. T., and Fett-Neto, A. G. 2009. Bioactive alkaloids from South American *Psychotria* and related species. *Open Bioactive Compd. J.* 2:29-36.
- Scholler, M., Abbani, M., and Friedrich, F. 2014. *Tranzschelia* in the Americas revisited: Two new species and notes on the *Tranzschelia thalictri* complex. *Mycologia* 106:448-455.
- Smith, W. H. 1970. *Tree Pathology—A Short Introduction*. Academic Press Inc., New York.
- Stevens, W. D., Ulloa-U, C., Pool, A., and Montiel, O. M. 2001. Flora of Nicaragua, angiosperms. Pages 1911-2666 in: *Monographs in Systematic Botany*. W. D. Stewans, U. Ulloa, P. Carmen, M. Amy, and M. Olga, eds. Missouri Botanical Garden Press, St. Louis.
- Thirumalachar, M. J., and Mundkur, B. B. 1949. Genera of rusts. *Indian Phytopathol.* 2:65-101.
- Thirumalachar, M. J., and Mundkur, B. B. 1950. Genera of rusts. *Indian Phytopathol.* 3:4-42.
- White, T. J., Bruns, T., Lee, S., and Taylor, J. W. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pages 315-322 in: *PCR Protocols: A Guide to Methods and Applications*. M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White, eds. Academic Press, Inc., New York.