Identification of *Nyssopsora thwaitesii* on *Schefflera* in northeast India

P. Baiswar · S. V. Ngachan · S. Chandra

Received: 26 July 2013 / Accepted: 24 January 2014 / Published online: 13 February 2014
© Australasian Plant Pathology Society Inc. 2014

Abstract During a routine survey rust was found on *Schefflera wallichiana* in Meghalaya in November–December 2012. Microscopic studies with light and scanning electron microscopy revealed the pathogen to be *Nyssopsora thwaitesii*. Molecular characterisation of the pathogen was also done by sequencing (18S), ITS region and large subunit (25S) of nrDNA of *N. thwaitesii*. This is the first identification of this pathogen on *S. wallichiana* from northeast India.

Keywords *Schefflera wallichiana* · *Nyssopsora thwaitesii* · *Tuberculina* sp · Hyperparasite · LSU · SSU · ITS

Schefflera wallichiana is used as fodder for animals and locally known as *Parari* grass. Synonyms include *Aralia wallichiana*, *Heptapleurum wallichianum* and *Paratropia wallichiana* (The Plant List 2010). Rust was observed on *Schefflera wallichiana* during November and December, 2012 in Barapani, Meghalaya, India. Rust pustules appeared as black powdery masses on both leaf surfaces, covering large areas of up to 3 cm with yellowish margins (Fig. 1). Spots were raised in most of the cases. Both older and younger leaves appeared to be equally susceptible.

Measurements were made after mounting spores in 3 % KOH at 1,000× using an Olympus BX 53 microscope equipped with a digital camera DP 72 (Olympus) and image analysis software (cellSens Standard 1.5, Olympus).

Scanning electron microscopy was conducted by sputter coating the dried specimens in a JFC-1100 fine coat ion sputter device (JEOL, Tokyo, Japan) under vacuum and then mounted on stubs and observed at 20 kV in JEOL JSM 6360 scanning electron microscope (JEOL, Tokyo, Japan).

For confirmation of generic identity 18S (SSU), ITS region and 25S (LSU) regions of nrDNA was also used. DNA isolation was done using the Qiagen stool kit. Primers (NS5, NS 7 and 5'-TCAACAGACTTGTACATGGTCCA-3' (a minor modification of ITS4BR)) were used for amplification in a nested PCR (first round NS 5 and modified ITS4BR and second round with NS 7). Cycling conditions had initial denaturation 5 min (94 °C), denaturation 30s (94 °C), annealing 50s (55–50 °C, touchdown with 1 °C decrement in every cycle up to 6 cycles), extension 1:20 min (72 °C) and final extension 5 min (72 °C) (38 cycles). For amplification of the 25S (LSU) region of the *Nyssopsora* sp., the nested PCR was done using primers 5'-GGACCATGTACAAGTCTGTTGA-3' (ITS4BrF) and 5'-CCTGAGGGAACTTCG-3' (LR 5) for the first round and ITS4BrF and 5'-CCGTGTTTCAAGACGGG -3' (LR 3) for the second round. Amplified products were checked through electrophoresis using ethidium bromide. Similarity searches were done using Blastn at NCBI.

Pathogen description: Spermagonia, aecia and uredinia unknown. Telia amphigenous, subepidermal in origin, erumpent, blackish, in large groups measuring 130–460 µm (Fig. 2a). Teliospores chestnut brown when mature, three celled, triquetrous and strongly constricted at the septum, measuring 27–42×22–35 µm, wall thickness 1–3 µm (Fig. 2b, c). Young pale cinnamon brown teliospores often mixed with mature ones. One to two germ pores in each cell of the teliospore. Projections up to 15 and branched at the tips measuring 11–14×5–7 µm.

P. Baiswar (✉) · S. V. Ngachan · S. Chandra
ICAR Research Complex for NEH Region,
Umiam 793103, Meghalaya, India
e-mail: pbaiswar@yahoo.com

Fig. 1 Symptoms of rust on *Schefflera wallichiana*

Pedicle persistent, hyaline measuring $35\text{--}52 \times 5\text{--}7\text{ }\mu\text{m}$ (Fig. 2d). Samples have been deposited in the herbarium collection of MACS Agharkar Research Institute, Pune (AMH 9528).

Generic characters of *Nyssopsora*—Spermatophytes unknown. Aecia are uredinoid if present without paraphyses. Aeciospores echinulate. Uredinia subepidermal, erumpent. Telia subepidermal. Teliospores three celled with conspicuous

projections and germ pores 1–4 (Cummins and Hiratsuka 2003). Therefore, based on 3-celled teliospores with conspicuous projections, we assigned our pathogen to *Nyssopsora*.

Nyssopsora sp. has been reported on Anacardiaceae, Araliaceae, Meliaceae, Naucleaceae, Pittosporaceae, Sapindaceae and Umbelliferae. In the Araliaceae family, *N. asiatica* (on *Aralia* and *Schefflera*), *N. clavellosa* (on *Aralia*), *N. thwaitesii* (*Brassiopsis*, *Hedera*, *Schefflera*), *N. trevesiae* (*Trevesia*), and *N. thirumalacharii* (on *Brassiopsis griffithii*) are reported. *Nyssopsora thwaitesii* has been reported on *S. bengalensis*, *S. stellata*, *S. roxburgii* and *S. venulosum* (Monson 1974; Ngachan and Goswami 1985; Bagyanarayana et al. 1987; Lohsomboon et al. 1990).

Our identification is based on the presence of telia on both leaf surfaces (amphigenous) and teliospores strongly constricted at the septum. *Nyssopsora thwaitesii* differs from *N. trevesiae* in having projections branched at the tips, from *N. clavellosa* in having strong septal constriction in teliospores, from *N. asiatica* in having amphigenous telia, from *N. thirumalacharii* by the presence of furcated projections (type specimen was also obtained from Kew (M) for observations on projections). *N. cedraleae* and *N. koelreuteriae* are not reported on Araliaceae. *Nyssopsora cedraleae*, *N. koelreuteriae* and *N. formosana* are not reported on Araliaceae; *N. cedraleae* and *N. formosana* also having aeciospores. *Nyssopsora schefflerae* is now considered

Fig. 2 **a** Telia of *Nyssopsora thwaitesii* on *Schefflera wallichiana* (Bar=200 μm) **b** Teliospores of *Nyssopsora thwaitesii* (Bar=10 μm) **c** Teliospores of *Nyssopsora thwaitesii* (Bar=100 μm) **d** Teliospore of *Nyssopsora thwaitesii* (Bar=10 μm) **e** Furcated projections on teliospore of *Nyssopsora thwaitesii* (Bar=2 μm)

Fig. 3 Parasitised pustules of rust (*Nyssopsora thwaitesii*) by *Tuberculina* sp.

synonymous with *N. thwaitesii* (Monson 1974; Ngachan and Goswami 1985; Bagyanarayana et al. 1987; Lohsomboon et al. 1990). Based on these facts we conclude that the species under consideration is *N. thwaitesii*.

Sequences of the SSU and ITS region (KF850482) and 25S (LSU) region (KF550283) have been deposited in Genbank. Blastn search using SSU and ITS region revealed 96 % identity with AY123312.1 and U77061.1 sequences of *N. echinata*. These are the only two sequences of *Nyssopsora* available in

Genbank. Blastn search using 25S (LSU) (KF550283) revealed 93 % identity with *Gymnosporangium* sp. since currently no LSU sequence of *Nyssopsora* is available in Genbank. Our sequence (KF550283) is the first sequence of LSU nrDNA of *N. thwaitesii* deposited in Genbank.

We also observed during our study that a few rust pustules (teliosori) were also parasitised by *Tuberculina* sp. also known as purple mold. Parasitised pustules had a tinge of violet colour (Fig. 3). SEM studies revealed that whole pustules were parasitized by *Tuberculina* sp. with profuse sporulation (Fig. 4). Microscopic studies were conducted as stated above using light and scanning electron microscope. Conidial dimensions were 9–13 μm . The sequence containing 18S (SSU), ITS region and large subunit of nrDNA has been deposited in GenBank (KC884701). Blastn search revealed maximum identity with *Helicobasidium mompa* (93 % with sequences AY292428 and AY292429). Many *Tuberculina* sp. are considered to be anamorphs of *Helicobasidium* sp., parasites of rust and also closely related to rusts (Lutz et al. 2004a, b).

This is the first identification of *N. thwaitesii* on *S. wallichiana* from India. *Nyssopsora thwaitesii* is reported on *S. bengalensis*, *S. stellata*, *S. roxburgii* and *S. venulosum* in mainland (central and southern parts of India) but not from northeast India nor neighbouring Myanmar, Bangladesh and Bhutan (Farr and Rossman 2012). It is of economic significance since this plant is used as fodder in this region.

Fig. 4 **a** Parasitised pustules of rust (*Nyssopsora thwaitesii*) by *Tuberculina* sp. (Bar=100 μm) **b** Non-parasitised pustules of rust (*Nyssopsora thwaitesii*) by *Tuberculina* sp. (Bar=50 μm) **c** Conidia of *Tuberculina* sp. (Bar=5 μm) **d** Conidia of *Tuberculina* sp. on teliospore of *Nyssopsora thwaitesii*

Acknowledgments Animal Production (including Poultry Science) is thanked for providing facilities for molecular work. Authors would like to thank Head SAIF, Dr Sudeep Dey (Scientific Officer), N. K. Rynjah, and Dr R. Charkraborty for scanning electron microscopy at Northeastern Hill University, Shillong, Meghalaya, India. Authorities at Kew K(M), England are thanked for loan of type specimen of *N. thirumulacharii*. Microscope (Olympus BX 53) used has been procured under National Initiative on Climate Resilient Agriculture (NICRA) project (PI, Dr DJ Rajkhowa). Dr P Singh, Animal Nutrition is also thanked for information on host plant.

References

- Bagyanarayana G, Subbalakshmi G, Ramachar P, Hosagoudar VB (1987) *Nyssopsora schefferae* sp. nov. from India. Curr Sci 56:1022–1023
- Cummins GB, Hiratsuka Y (2003) Illustrated genera of rust fungi, 3rd edn. APS Press, St. Paul
- Farr DF, Rossman AY (2012) Fungal databases, systematic botany & mycology laboratory, USDA: ARS. Available at <http://nt.ars-grin.gov/fungaldatabases> (Retrieved December 7, 2012)
- Lohsomboon P, Kakishima M, Ono Y (1990) A revision of the genus *Nyssopsora* (Uredinales). Mycol Res 94:907–922
- Lutz M, Bauer R, Begerow D, Oberwinkler F (2004a) *Tuberculina-Thanatophytum/Rhizoctonia crocorum-Helicobasidium*: a unique mycoparasitic-phytoparasitic life strategy. Mycol Res 108:227–238
- Lutz M, Bauer R, Begerow D, Oberwinkler F, Triebel D (2004b) *Tuberculina*: rust relatives attack rusts. Mycologia 96:614–626
- Monson HL (1974) The species of *Triphragmium*, *Nyssopsora*, and *Triphragmiopsis*. Mycopathol Mycol Appl 52:115–131
- Ngachan SV, Goswami RN (1985) *Nyssopsora thirumulacharii* - a new rust from India. Indian Phytopathol 38:186–187