



***Nyssopsoraceae*, a new family of *Pucciniales* to accommodate *Nyssopsora* spp.**

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Abstract

A new species of rust fungi *Nyssopsora toonae* discovered on living leaves of *Toona sinensis* (\equiv *Cedrela sinensis*) from Uttarakhand, India, is described and illustrated. *N. cedrelae* is also reported on the same host plant, but differs from *N. toonae* which has a wide range of cells (1–4) and diverse teliospores shapes. Such features are not reported in any other species of the *Nyssopsora*. In a phylogenetic analyses based on partial 28S large subunit (LSU), 18S smaller subunit (SSU), cytochrome c-oxidase subunit 3 (CO3) and complete internal transcribed spacer (ITS) sequence data, all the *Nyssopsora* spp. along with *N. toonae* clustered together and form a separate and independent monophyletic lineage sister to *Pucciniaceae* in *Pucciniales*. The new family *Nyssopsoraceae* is introduced to accommodate this lineage based on the phylogenetic evidence and morphological differences from other known families. *Nyssopsoraceae* is characterised by its teliospores borne singly on a pedicel, with simple or branched projections all over the surface, composed of 1–4 cells (mostly 3-celled), and diverse shape. The teliospores spherical to subspherical (1-celled), dumbbell (2-celled), linearly arranged to triquetrous (3-celled), or T-shaped to tetrahedron (4-celled). A comparison of the morphological features, host plants and geographical distribution of all validly accepted species of *Nyssopsora* is provided.

Keywords – Phylogeny – Plant pathogenic – Rust fungi – Taxonomic novelty

Introduction

The rust fungi (*Pucciniales*) are obligate phyto-pathogens and represent one of the largest orders in Basidiomycota. They are a monophyletic group with more than 7000 species (Kirk et al. 2008) distributed globally. Diseases caused by rust fungi have drastically impacted human agriculture and history through time. These fungi have a broader host range from pteridophytes (ferns) to higher plants including gymnosperms and angiosperms, although individual species usually have a restricted host range. Rust fungi can have a microcyclic and autoecious life cycle to a macrocyclic life cycle with up to five spore states. Heteroecious species requires two unrelated hosts, although the life cycle can be modified and reduced (Cummins & Hiratsuka 2003). From India, 640 species of rust fungi have been recorded belonging to 69 genera and 16 families (Gautam et al. 2021).

Initially, rust fungi were classified into families based on characteristics of basidia and teliospores (Cunningham 1931). Later, teliospores were the most important spore state to distinguish genera and families in the classical taxonomy of *Pucciniales* (Cummins & Hiratsuka 2003, Maier et al. 2003, Aime 2006, van der Merwe et al. 2007, Beenken & Wood 2015). Arthur (1906) established the genus *Nyssopsora* with *N. Echinata* (Lév.) Arthur as the type species. *Nyssopsora* is characterized by 3-celled teliospores with a basal pedicellate cell surmounted by two further cells and bearing conspicuous projections (spines) on its surface. Both Lütjeharms (1937) and Lohsomboon et al. (1990) have monographed the genus *Nyssopsora*. *Nyssopsora* was originally included in *Triphragmium* Link (1825). The main characteristics of *Triphragmium* were subglobose, 3-celled, pedicellate teliospores divided by transverse and longitudinal septa and with subepidermal telia. Both *Nyssopsora* and *Triphragmium* were originally assigned to *Sphaerophragmiaceae* Cummins & Y. Hirats. because of their three celled teliospores with triangular arrangement (Cummins & Hiratsuka 1983).

Cummins & Hiratsuka (1983) established *Sphaerophragmiaceae*, covering the genera *Cumminsina* Petr., *Hapalophragmium* Syd. & P. Syd. (= *Hapalophragmiopsis* Thirum., = *Triactella* Syd.), *Hennenia* Buriticá, *Nyssopsora* Arthur (= *Oplophora* Syd.), *Sphaerophragmium* Magnus, *Triphragmiopsis* Naumov (= *Nyssopsorella* Syd.) and *Triphragmium* Link. These genera were mainly based on pedicellate teliospores with three or more spherically arranged cells. The connections between *Nyssopsora* and *Sphaerophragmium* were noted by Lohsomboon et al. (1994). Cummins & Hiratsuka (2003) merged the *Sphaerophragmiaceae* into *Raveneliaceae* Leppik. McTaggart et al. (2016) showed that the type species of *Sphaerophragmium*, *S. acacieae* (Cook) Magnus, was separated from *Raveneliaceae*. Therefore, *Sphaerophragmiaceae* was re-erected by McTaggart et al. (2016). *Sphaerophragmiaceae* did not contain any genera except *Sphaerophragmium* in the original description by Cummins & Hiratsuka (1983). Currently *Sphaerophragmiaceae* consists of *Austropuccinia* Beenken, *Dasyspora* Berk. & M.A. Curtis, *Puccorchidium* Beenken, *Sphenorchidium* Beenken and *Sphaerophragmium* (Beenken 2017, Aime & McTaggart 2020). The current status of *Nyssopsora* is not resolved and it remains *incertae sedis* within *Uredinineae*, recovered as sister to *Sphaerophragmiaceae* (Aime & McTaggart 2020).

Based on multi-gene sequence analysis, a stable and resolved higher-rank classification for the rust fungi (*Pucciniales*) was given by Aime & McTaggart (2020) that comprising 7 suborders and 18 families. Aime & McTaggart (2020) discussed the evolutionary trends that led to diversification and current status within *Pucciniales*, but some families/genera *Pucciniastrum* and *Pucciniastraceae*; *Raveneliaceae*; and *Allodus*, *Neopuccinia*, and *Nyssopsora*, could not be confidently resolved.

In December 2018, a survey was conducted to explore the diversity of phytopathogenic fungi in Chamoli district of Uttarakhand, India. During this survey, an interesting rare rust fungus was discovered on living leaves of *Toona sinensis* (Juss.) M. Roem. that caused blackish brown pustules as rusty masses restricted to the lower surface of leaves. This fungus was identified as a new species of *Nyssopsora* due to the presence of 1–4-celled (mostly 3-celled) pedicellate teliospores bearing conspicuous projections on its surface. There are 12 validly accepted species of *Nyssopsora* (www.indexfungorum.org and www.mycobank.org; accessed 15 September, 2023). In light of the above, the present study focused on the phylogenetic analyses using more species and genera including *Nyssopsora* and *Triphragmium*, to find out exact placement of all the *Nyssopsora* spp. under *Pucciniales*. The present communication provides a comparative account of major morphological features, spore states, hosts and geographical distribution of the validly accepted species of *Nyssopsora* (Tables 1, 2).

Materials & Methods

Isolates and morphology

During a survey an interesting rare foliicolous rust was encountered on *Toona sinensis* from Uttarakhand, India, in December 2018. The infected leaves were collected in sterile polybag in

between blotting papers and brought to the laboratory along with collection details. Close-up photographs of infection spots for different developmental stages of spores were captured using camera (CatCam300EF) attached to a Magnus Stereo Zoom Trinocular Olympus Microscope (MSZ-TR). Slides were prepared by hand sectioning and scraping from infection spots of freshly collected leaves and mounted in both lactophenol cotton-blue mixture and 50% glycerine. Fungal propagules were photographed using an Olympus compound microscope (CH20i-TR) equipped with Magnus camera (MIPS CMOS). Scanning electron microscopy (SEM) was done with a Carl Zeiss EVO 18. Detailed observations of morphological characters were carried out at different magnifications through light microscopy (450× and 1000×) and scanning electron microscopy (up to ~35K×). For SEM micrographs specimens were coated with gold-palladium using a POLARON Sputter coater (180 sec in nitrogen atmosphere of 20 mA, 30 mm distance from the electrode) and examined with SEM. Measurements were made for 25 of each morphological feature of urediniospores and teliospores. Holotype material is deposited in the Ajrekar Mycological Herbarium (AMH), Agharkar Research Institute (ARI), Pune, India and isotype material is retained in the Mycological Herbarium of the Department of Botany of Banaras Hindu University, Varanasi, U.P., India (MH-BHU).

DNA extraction

Genomic DNA was extracted from spores scrapped from the heavily infected surface of leaves using a sterile scalpel blade. Harvested spores and mycelium about 150 mg was transferred to a 2 ml polypropylene centrifuge tube kept in liquid nitrogen for 2 min and then grinded to make fine powder using a mortar and pestle. From powdered form, DNA was isolated using Himedia DNA isolation Kit (HiPurA™ Fungal DNA Purification Kit) following the manufacturers' protocols. Isolated DNA fragments were visualised by electrophoresis in 1% agarose gel (w/v) stained with ethidium bromide under Gel Documentation system (Bio-Rad Universal Hood II) and DNA concentration was quantified by using Nano Drop microvolume spectrophotometer (ThermoScientific™ NanoDrop™ One/One^C Microvolume UV-Vis Spectrophotometer with Wi-Fi).

Polymerase chain reaction (PCR) and sequencing

The internal transcribed spacer (ITS) region, large subunit (LSU) and smaller subunit (SSU) of nrDNA were amplified using Rust2inv (5'-GATGAAGAACACAGTGAAA-3')/ITS4rust (5'-CAGATTACAAATTTGGGCT-3') (Aime 2006, Beenken et al. 2012), LROR (5'-ACCCGCTGAACTTAAGC-3')/LR6 (5'-CGCCAGTTCTGCTTACC-3') (Vilgalys & Hester 1990) and NS1 (5'-GTAGTCATATGCTTG TCTC-3')/NS4 (5'-CTTCCGTCAATTCCTTTAAG-3') (White et al. 1990) primer pairs, respectively. PCRs were carried out in 50 µL reaction mixture containing 5 µL Taq buffer containing MgCl₂, 1 µL dNTPs (10 mM), 1 µL each forward and reverse primer (10 µmol/µL), 5 µL of DNA template (~35 ng/µL), 0.3 µL of Taq DNA polymerase (5 Unit/µL) and 36.7 µL of milli-Q water. The PCRs were carried out in Thermal Cycler (Bio-Rad T100™). Conditions for the PCRs amplification consisted of an initial denaturation at 95 °C for 5 min; followed by 35 cycles of denaturation at 94 °C for 1 min; annealing at 53 °C for ITS, 65 °C for LSU and 55 °C for SSU for 1 min, extension at 72 °C for 1 min. The final extension step was done at 72 °C for 8 min. The amplified amplicons were run in 1.2% agarose gel and visualised in the Gel Documentation system (Bio-Rad Universal Hood II) for the product size and purity. PCR products were cleaned and sequenced with the amplification primers at AgriGenome Labs Private Ltd., Kerala by the Sanger sequencing method using BigDye® Terminator v3.1 Cycle sequencing Kit and ABI 3100 DNA analyzer.

Phylogenetic analysis

The obtained ITS, LSU and SSU sequences from type materials were assembled and edited with Chromas v.2.6.6. The manually edited sequences were submitted to NCBI GenBank (Table 1) and were subjected to a megablast search of the NCBI GenBank nucleotide database. The most

homologous sequences of related strains were retrieved. Reference sequences were also selected based on sequence availability from relevant published literature (Table 1). Sequence alignments were generated using MAFFT v.6.864b (Kato & Toh 2010) whereas BioEdit v.7.0.9 (Hall 2007) and MEGA-X v.10.1.8 (Kumar et al. 2018) were used to manually check, improve and concatenate the aligned sequences. Sequence alignments were deposited as electronic supplementary materials in TreeBASE, study number 29662.

The phylogenetic methods used in this study included a Bayesian analysis (BI) performed with MrBayes v.3.2.7 (Ronquist et al. 2012) and maximum likelihood (ML) analysis performed with RAxML v.8.2.10 (Stamatakis 2014). The phylogenetic analyses were individually applied to the two datasets: dataset 1 consisted of concatenated alignments of LSU, SSU and CO3 sequences whereas dataset 2 consisted of concatenated alignments of LSU, SSU, CO3 and ITS sequences from 18 families currently belonging to *Pucciniales*. The sequences of taxa containing weak aligned portions, incomplete data, missing sequence data and gaps were removed. The overview trees were rooted with *Eocronartium muscicola*, from the sister order to *Pucciniales* (Aime et al. 2006).

Bayesian inference was implemented with the GTR+I+G model. Bayesian inference was calculated using a Markov chain Monte Carlo (MCMC) algorithm with Bayesian posterior probabilities (Rannala & Yang 1996). The analysis was performed up to 2300000 generations till the standard deviation of split frequency came down below 0.01. The first 25% of generated trees representing the burn-in phase were discarded, and the remaining trees were used to calculate posterior probabilities of the majority rule consensus tree. ML analysis was also performed using a GTR model of site substitution, including GAMMA+P-Invar model of rate heterogeneity and a proportion of invariant sites (Stamatakis 2014). The ML support values were evaluated with a bootstrapping method of 1000 replicates. These analyses involved 102 nucleotide sequences.

Presented trees were obtained with the ML approach. Tree reconstruction, visualization and editing were done using FigTree v.1.4.4 and TreeGraph_2.15.0. The multigene phylograms are shown in Figs 9, 10.

Results

The data for the trees conducted in different analyses are shown in Table 1. Phylogenetic trees obtained from combined genes analyses are supplied below.

Dataset 1 (LSU, SSU and CO3 phylogeny)

This dataset consisted of a concatenated alignment of three loci (LSU, SSU and CO3). The final alignment contained a total of 1987 characters divided into three partitions containing 1006 (LSU), 437 (SSU) and 544 (CO3) characters, respectively including the alignment gaps. Phylogenetic trees generated from Bayesian analyses (BI) and maximum likelihood (ML) produced trees with overall similar topology. A best scoring RAxML tree is presented in Fig. 9, with the likelihood value of -23481.773327 .

In this analysis, the exact placement of *Nyssopsora* spp. that were earlier not resolved, clustered together and recovered as sister to *Pucciniaceae* and delineate a separate independent lineage with strong bootstrap support within *Pucciniales*. The ML tree (Fig. 9) was mostly congruent with prior studies of more limited taxon and locus sampling (Aime 2006, Beenken & Wood 2015, McTaggart et al. 2016, Beenken 2017, Aime et al. 2017, 2018, Souza et al. 2018).

Dataset 2 (LSU, SSU, CO3 and ITS phylogeny)

This dataset consisted of a concatenated alignment of four loci (LSU, SSU, CO3 and ITS). The final alignment of this dataset contained a total of 2662 characters divided into four partitions containing 1006 (LSU), 437 (SSU), 544 (CO3) and 675 (ITS) characters, respectively including the alignment gaps. Phylogenetic trees generated from Bayesian analyses and maximum likelihood (ML) produced trees with an overall similar topology. A best scoring RAxML tree is presented (Fig. 10), with the likelihood value of -31766.255887 .

Table 1 Taxa included in molecular phylogenetic analyses and their GenBank accession numbers. The sequences in bold were generated in this study.

Taxon	Voucher ID	GenBank accession no.				Host	Source
		28S	18S	CO3	ITS		
Araucariomycetaceae							
<i>Araucaromyces fragiformis</i>	BRIP 68996	MW049245	MW049292	MW036497	NA	<i>Agathis robusta</i>	Aime & McTaggart (2020)
Coleosporiaceae							
<i>Chrysomyxa arctostaphyli</i>	CUW CFB 22246	AF522163	AY657009	NA	NA	NA	Aime & McTaggart (2020)
<i>Chrysomyxa reticulata</i>	PDD 92535	KX985767	NA	NA	KX985767	<i>Rhododendron</i> sp. sect. <i>Vireya</i>	Padamsee & McKenzie (2017)
<i>Chrysomyxa rhododendri</i>	PDD 102088	KJ698630	KJ746824	NA	NA	<i>Rhododendron</i> sp.	Padamsee & McKenzie (2014)
<i>Coleosporium inulae</i>	BPI 871127	MG907223	NA	NA	NA	<i>Inula fragilis</i>	Aime et al. (2018)
<i>Coleosporium melampyri</i>	PUR N16579	MG907224	NA	NA	NA	<i>Rhinanthus aristatus</i>	Aime et al. (2018)
<i>Coleosporium senecionis</i>	PDD 98309	KJ716348	KJ746818	NA	KJ716348	<i>Senecio</i> sp.	Padamsee & McKenzie (2014), Aime & McTaggart (2020)
<i>Coleosporium tussilaginis</i>	PUR N16713	MG907228	NA	NA	MG907228	<i>Sonchus</i> sp.	Aime et al. (2018)
<i>Thekopsora areolata</i>	NA	KJ546894	NA	NA	NA	<i>Picea engelmannii</i>	Aime & McTaggart (2020)
Crossosporaceae							
<i>Crossospora fici</i>	BRIP 58118	MH047207	MH047212	MH047204	NA	<i>Ficus virens</i> var. <i>sublanceolata</i>	Aime & McTaggart (2020)
<i>Crossospora ziziphi</i>	BPI 877877	MG744558	NA	NA	NA	<i>Ziziphus mucronata</i>	Souza et al. (2018)
<i>Kweilingia bambusae</i>	PUR F18200	MW147026	NA	NA	NA	<i>Bambusa</i> sp.	Aime & McTaggart (2020)
Gymnosporangiaceae							
<i>Gymnosporangium clavariiforme</i>	BRIP 59471	MW049261	MW049296	MW036499	NA	<i>Crataegus</i> sp.	Aime & McTaggart (2020)
<i>Gymnosporangium gracile</i>	20140326-1-GR-P25	KM486544	NA	NA	KM486542	<i>Juniperus oxycedrus</i>	Fernandez & Alvarado (2016)
Melampsoraceae							
<i>Melampsora abietis-populi</i>	HMAS 247978	MK064529	NA	NA	MK028579	<i>Populus wilsonii</i>	Zheng et al. (2019)

Table 1 Continued.

Taxon	Voucher ID	GenBank accession no.				Host	Source
		28S	18S	CO3	ITS		
<i>Melampsora epitea</i>	DAOM 240968	HQ317514	NA	NA	HQ317514	<i>Salix candida</i>	Liu et al. (2015)
<i>Melampsora euphorbiae</i>	BPI 863501	DQ437504	DQ789986	MW036501	NA	<i>Euphorbia macroclada</i>	Aime (2006), Matheny et al. (2006), Aime & McTaggart (2020)
<i>Melampsora hypericorum</i>	PDD 97325	KJ716353	KJ746828	NA	KJ716353	<i>Hypericum androsaemum</i>	Padamsee & McKenzie (2014)
<i>Melampsora laricis-populina</i>	HMAS 247976	MK064525	NA	NA	MK028584	<i>Populus simonii</i>	Zhenget al. (2019)
<i>Melampsora</i> sp.	SAL103	EF192205	NA	NA	NA	<i>Salix amygdaloides</i>	Bennett et al. (2011)
Milesiaceae							
<i>Milesia polypodii</i>	KR-M-0043190	MK302190	NA	NA	MH908415	<i>Polypodium vulgare</i>	Bubner et al. (2019)
<i>Milesina kriegieriana</i>	KR-M-0048480	MK302207	NA	NA	MH908452	<i>Dryopteris dilatata</i>	Bubner et al. (2019)
<i>Milesina philippinensis</i>	BRIP 58421	KM249868	NA	NA	NA	<i>Nephrolepis</i> sp.	McTaggart et al. (2014)
<i>Milesina thailandica</i>	IBAR 11436	LC498526	NA	NA	LC498526	<i>Lygodium flexuosum</i>	Onoet al. (2020)
<i>Milesina vogesiaca</i>	PURN659a	MG907235	NA	NA	NA	<i>Polystichum aculeatum</i>	Aime et al. (2018)
<i>Naohidemycetes vaccinii</i>	BPI 871754	DQ354563	DQ354562	NA	NA	<i>Vaccinium ovatum</i>	Aime (2006)
<i>Uredinopsis osmundae</i>	BPI 872258	MG907245	NA	NA	NA	<i>Osmunda claytoniana</i>	Aime et al. (2018)
Nyssopsoraceae							
<i>Nyssopsora echinata</i>	KR-0012164 (U1022), ESS244	MW049272	U77061	NA	NA	<i>Meum athamanticum</i>	Aime & McTaggart (2020)
<i>Nyssopsora koelreuteriae</i>	BBSW-1	NA	NA	NA	KT750965	<i>Koelreuteria bipinnata</i>	Yang et al. (2016)
<i>Nyssopsora thwaitesii</i>	AMH 9528	KF550283	NA	NA	KF550283	<i>Schefflera wallichiana</i>	Baiswar et al. (2014)
<i>Nyssopsora toonae</i>	AMH 10124	MT712660	ON641038	NA	MT712662	<i>Toona sinensis</i>	Present study
Ochropsoraceae							
<i>Aplopsora nyssae</i>	BPI 877823	MW049244	NA	NA	NA	<i>Nyssa sylvatica</i>	Aime & McTaggart (2020)
<i>Ochropsora ariae</i>	KR-0015027	MW049273	NA	NA	NA	<i>Anemone nemorosa</i>	Aime & McTaggart (2020)
Phakopsoraceae							
<i>Cerotelium fici</i>	UACH107	MF580676	NA	NA	NA	<i>Ficus carica</i>	Solano-Báez et al. (2017)

Table 1 Continued.

Taxon	Voucher ID	GenBank accession no.				Host	Source
		28S	18S	CO3	ITS		
<i>Masseëlla capparisi</i>	BRIP 56844	JX136798	NA	KT199413	NA	<i>Flueggea virosa</i>	McTaggart et al. (2016)
<i>Phakopsora myrtacearum</i>	PREM 61155	KP729473	NA	KT199414	KP729468	<i>Eucalyptus grandis</i>	Maier et al. (2016), McTaggart et al. (2016)
<i>Phakopsora pachyrhizi</i>	BRIP 56941	KP729475	MW049300	MW036503	NA	<i>Neonotonia wightii</i>	Maier et al. (2016), Aime & McTaggart (2020)
<i>Phragmidiaceae</i>							
<i>Gerwasia rubi</i>	BRIP 58369	KT199397	NA	KT199408	NA	<i>Rubus</i> sp.	McTaggart et al. (2016)
<i>Gymnoconia interstitialis</i>	BPI 747600	JF907677	DQ521422	NA	NA	<i>Rubus allegheniensis</i>	Yun et al. (2011), Aime & McTaggart (2020)
<i>Gymnoconia peckiana</i>	BPI 879271	GU058010	NA	NA	GU058010	<i>Rubus</i> sp.	Dixon et al. (2010)
<i>Hamaspora acutissima</i>	BRIP 55606	KT199398	KT199385	KT199409	NA	<i>Rubus moluccanus</i>	McTaggart et al. (2016)
<i>Kuehneola uredinis</i>	BPI 871104	DQ354551	DQ092919	NA	DQ354551	<i>Rubus argutus</i>	Aime (2006), Aime & McTaggart (2020)
<i>Phragmidium barnardii</i>	BRIP 56945	KT199402	NA	KT199415	NA	<i>Rubus multibracteatus</i>	McTaggart et al. (2016)
<i>Phragmidium sanguisorbae</i>	BPI 872232	JF907674	NA	NA	NA	<i>Sanguisorba minor</i>	Yun et al. (2011)
<i>Phragmidium tormentillae</i>	BPI 843392	DQ354553	DQ354552	MG907265	MG907214	<i>Potentilla canadensis</i>	Aime (2006), Aime et al. (2018)
<i>Phragmidium violaceum</i>	BPI 879276	GU058015	NA	NA	GU058015	<i>Rubus parviflorus</i>	Dixon et al. 2010
<i>Trachyspora intrusa</i>	BPI 843828	DQ354550	DQ354549	MW036508	DQ354550	<i>Alchemilla vulgaris</i>	Aime (2006), Aime & McTaggart (2020)
<i>Triphragmium ulmariae</i>	BPI 881364	JF907676	AY125401	NA	NA	<i>Filipendula ulmaria</i>	Wingfield et al. (2004), Yun et al. 2011
<i>Pileolariaceae</i>							
<i>Pileolaria brevipes</i>	PUR N16525, BPI 877989	MG907216	MW049301	MG907267	NA	<i>Toxicodendron</i> sp.	Aime et al. (2018), Aime & McTaggart (2020)
<i>Pileolaria pistaciae</i>	PURN11945	KY314266	NA	NA	MG860928	<i>Pistacia chinensis</i>	Ishaq et al. (2020)

Table 1 Continued.

Taxon	Voucher ID	GenBank accession no.				Host	Source
		28S	18S	CO3	ITS		
<i>Pileolaria toxicodendri</i>	BPI 871761	DQ323924	NA	NA	NA	<i>Toxicodendron</i> sp.	Scholler & Aime 2006, Aime & McTaggart (2020)
<i>Pucciniaceae</i>							
<i>Aecidium kalanchoe</i>	BPI 843633	AY463163	DQ354524	NA	NA	<i>Kalanchoe blossfeldiana</i>	Hernandez et al. (2004), Aime (2006)
<i>Ceratocoma jacksoniae</i>	BRIP 57762	KT199394	KT199382	KT199405	NA	<i>Daviesia</i> sp.	McTaggart et al. (2016)
<i>Cumminsia mirabilissima</i>	BPI 871101	DQ354531	DQ354530	NA	NA	<i>Mahonia aquifolium</i>	Aime (2006)
<i>Endophylloides portoricensis</i>	BPI 844288	DQ354516	AY125389	NA	DQ354516	<i>Mikania micrantha</i>	Aime & McTaggart (2020)
<i>Leptopuccinia malvacearum</i>	BRIP 57522	KU296888	NA	KX999924	NA	<i>Malva parviflora</i>	Aime & McTaggart (2020)
<i>Miyagia pseudosphaeria</i>	BPI 842230	DQ354517	AY125411	NA	NA	<i>Sonchus oleraceus</i>	Aime & McTaggart (2020)
<i>Puccinia andropogonis</i>	BPI 871763	GU057993	NA	NA	NA	<i>Andropogon</i> sp.	Dixon et al. (2010)
<i>Puccinia coronate</i>	BPI 844300	DQ354526	DQ354525	NA	NA	<i>Rhamnus cathartica</i>	Aime (2006)
<i>Puccinia coronate</i> var. <i>avenae</i>	BRIP 57635	MW147047	NA	MW139657	NA	<i>Avena sativa</i>	Aime & McTaggart (2020)
<i>Puccinia graminis</i>	BRIP 60137	KM249852	MW049302	MW036505	NA	<i>Glyceria maxima</i>	Deadman et al. (2011), Aime & McTaggart (2020)
<i>Puccinia hordei</i>	BPI 871109	DQ354527	DQ415278	NA	NA	Unidentified Poaceae	Aime (2006), Aime et al. (2006)
<i>Puccinia platyspora</i>	BPI 091376	KT827311	NA	NA	NA	<i>Sphaeralcea</i> sp.	Demers et al. (2015)
<i>Puccinia porri</i>	BRIP 64600	KY849820	NA	NA	KY849820	<i>Allium porrum</i>	McTaggart et al. (2017)
<i>Puccinia sherardiana</i>	BPI 871783	KT827315	NA	NA	NA	<i>Sphaeralcea</i> sp.	Demers et al. (2015)
<i>Puccinia windsoriae</i>	BPI 871790	GU057995	NA	NA	NA	<i>Tridens</i> sp.	Dixon et al. (2010)
<i>Puccinosira solani</i>	RS25	EU851140	NA	NA	NA	<i>Solanum nigrum</i>	Zuluaga et al. (2011)
<i>Uromyces plumbarius</i>	NA	KP313731	NA	NA	KP313731	<i>Gaura lindheimeri</i>	Blomquist et al. (2015b)
<i>Pucciniastraceae</i>							
<i>Hyalopsora aspidiotus</i>	PUR N4641	MW049264	NA	NA	NA	<i>Gymnocarpium dryopteris</i>	Aime & McTaggart (2020)
<i>Melampsorium betulinum</i>	BPI 871107	DQ354561	AY125391	NA	NA	<i>Alnus</i> sp.	Wingfield et al. (2004), Aime (2006)

Table 1 Continued.

Taxon	Voucher ID	GenBank accession no.				Host	Source
		28S	18S	CO3	ITS		
<i>Pucciniastrum epilobii</i>	PUR N11088	MW049277	NA	NA	NA	<i>Epilobium angustifolium</i>	Aime & McTaggart (2020)
<i>Pucciniastrum minimum</i>	BRIP 57654	MG907242	KT199391	KT199422	NA	<i>Vaccinium corymbosum</i>	McTaggart et al. (2016), Aime et al. (2018)
Raveneliaceae							
<i>Diorchidium woodii</i>	U1475	MW111538	MW111533	NA	NA	<i>Millettia grandis</i>	Aime & McTaggart (2020)
<i>Endoraecium auriculiforme</i>	BRIP 56548	KJ862298	NA	KJ862432	KJ862355	<i>Acacia auriculiformis</i>	McTaggart et al. (2015)
<i>Endoraecium parvum</i>	BRIP 57524	KJ862316	KJ862409	KJ862445	KJ862369	<i>Acacia leiocalyx</i>	McTaggart et al. (2015)
<i>Kernkampella breyniae</i>	BRIP 56909	KJ862346	KJ862428	KJ862459	NA	<i>Breynia cernua</i>	McTaggart et al. (2015)
<i>Maravalia limoniformis</i>	BRIP 59649	MW049266	NA	MW036500	NA	<i>Austrostenisia blackii</i>	Aime & McTaggart (2020)
<i>Olivea scitula</i>	BPI 871108	DQ354541	DQ354540	NA	NA	<i>Vitex doniana</i>	Aime (2006)
<i>Porotenus biporus</i>	ZT Myc 3414	JF263494	JF263510	NA	NA	<i>Memora flavida</i>	Beenken et al. (2012)
<i>Prospodium tuberculatum</i>	BRIP 57630	KJ396195	KJ396196	MW036504	NA	<i>Lantana camara</i>	Pegg et al. (2014), Aime & McTaggart (2020)
<i>Ravenelia evansii</i>	PREM 61846	MG945993	NA	MN095321	MG945961	<i>Vachellia luederitzii</i> var. <i>retinens</i>	Ebinghaus & Begerow (2018), Ebinghaus et al. (2018, 2020)
Rogerpetersoniaceae							
<i>Rogerpetersonia torreyae</i>	BPI 877825, BPI 877824	MG907207	MG907197	MG907254	NA	<i>Torreya californica</i>	Aime et al. (2018)
Skierkaceae							
<i>Skierka diploglottidis</i>	BRIP 59646	MW049278	MW049304	MW036506	NA	<i>Dictyoneura obtusa</i>	Aime & McTaggart (2020)
<i>Skierka robusta</i>	BPI 879954	MW049279	MW049305	NA	NA	<i>Rhoicissus rhomboidea</i>	Aime & McTaggart (2020)
Sphaerophragmiaceae							
<i>Austropuccinia psidii</i>	BRIP 57793	KF318449	KF318457	KT199419	NA	<i>Rhodamnia angustifolia</i>	Pegg et al. (2014), McTaggart et al. (2016)
<i>Dasyscypha gregaria</i>	ZT Myc 3397	JF263477	JF263502	JF263518	JF263477	<i>Xylopiya cayennensis</i>	Beenken et al. (2012)
<i>Dasyscypha segregaria</i>	PMA MP4941	JF263488	JF263507	JF263523	JF263488	<i>Xylopiya aromatica</i>	Beenken et al. (2012)

Table 1 Continued.

Taxon	Voucher ID	GenBank accession no.				Host	Source
		28S	18S	CO3	ITS		
<i>Puccorchidium polyalthiae</i>	ZT HeRB 251	JF263493	JF263509	JF263525	JF263493	<i>Polyalthia longifolia</i>	Beenken et al. (2012)
<i>Puccorchidium popowiae</i>	ZT Myc 1976	JF263495	JF263511	JF263526	JF263495	<i>Monanthotaxis caffra</i>	Beenken et al. (2012)
<i>Sphaerophragmium acaciae</i>	BRIP 56910	KJ862350	KJ862429	KJ862462	NA	<i>Albizia</i> sp.	McTaggart et al. (2015)
<i>Sphenorchidium deightonii</i>	PC 0096730	KM217350	KM217368	NA	KM217350	<i>Xylopia aethiopica</i>	Beenken & Wood (2015)
<i>Sphenorchidium xylopiiae</i>	NA	KM217355	KM217372	NA	KM217355	<i>Xylopia aethiopica</i>	Beenken & Wood (2015)
Tranzscheliaceae							
<i>Tranzschelia discolor</i>	BRIP 57662	KR994891	KR994969	KR995082	NA	<i>Prunus persica</i>	Doungsa-ard et al. (2018)
<i>Tranzschelia mexicana</i>	KR-M-0040855	KP308391	NA	NA	KP308391	<i>Prunus salicifolia</i>	Blomquist et al. (2015a)
Uredinineae incertae sedis							
<i>Allodus podophylli</i>	BPI 842277, PUR N16753	DQ354543	DQ354544	MG907270	DQ354543	<i>Podophyllum peltatum</i>	Aime (2006), Aime et al. (2018)
Zaghouaniaceae							
<i>Achrotelium ichnocarpi</i>	BRIP 55634	KT199393	KT199381	KT199404	NA	<i>Ichnocarpus frutescens</i>	McTaggart et al. (2016)
<i>Blastospora smilacis</i>	PUR N270	DQ354568	DQ354567	NA	NA	<i>Smilax sieboldii</i>	Aime (2006)
<i>Hemileia vastatrix</i>	BRIP 61233	KT199399	DQ354565	KT199410	NA	<i>Coffea robusta</i>	Aime (2006), McTaggart et al. (2016)
<i>Mikronegeria fagi</i>	PUR N16373	MW049267	MW049298	NA	NA	<i>Nothofagus oblique</i>	Aime & McTaggart (2020)
<i>Mikronegeria fuchsiae</i>	PDD 101517	KJ716350	KJ746826	NA	KJ716350	<i>Phyllocladus trichomanoides</i>	Padamsee & McKenzie (2014)
<i>Zaghouanianotelaeeae</i>	BRIP 58325	KT199396	KT199384	KT199407	NA	<i>Notelaea microcarpa</i>	McTaggart et al. (2016)
Out group							
<i>Eocronartium muscicola</i>	MIN796447, DUKE:DAH(e1)	AF014825	DQ241438	NA	NA	NA	Bruns & Szaro Unpublished, Henk & Vilgalys et al. (2007)

Table 2 Morphological details of *Nyssopsora* species.

<i>Nyssopsora</i> spp.	Teliospores							Projecton/spores			Urediniospores		
	NC [†]	Size (µm)	Septal Constriction	Wall thickness (µm)	GP [†]	Pedicel (µm)	NP [†]	Length (µm)	TF [†]	Size (µm)	WT [†] (µm)	SO [†]	GP [†]
<i>asiatica</i>	3	26–41 × 26–42	Slight to moderate	1–3	1–2(3)	Up to 60 × 5–8	13–29	6–15	2–>5	–	–	–	–
<i>cedrelae</i>	3	29–44 × 27–44	Slight	1–3	1–3	Up to 105 × 7–12	13–27	3–9	2–3	14–24 × 13–21	1–2.5	E	NS
<i>citriobati</i>	3	28–39 × 28–38	Slight	1–3.5	1–3	Up to 40.5 × 3–5	14–19	2.5–6	2–6	–	–	–	–
<i>clavellosa</i>	3	29–37 × 26–38	Slight to moderate	1.5–3	1–4	Up to 94 × 5–9	10–23	4–11	3–4	–	–	–	–
<i>echinata</i>	3	29–42 × 27–40	Slight	1–3	1–4	Up to 56 × 4–8	14–23	4–18	1–3	–	–	–	–
<i>eocaenica</i>	3	33–38 × 34–37	Moderate	–	–	Up to 6–7	Numerous	–	0	–	–	–	–
<i>formosana</i>	3	27–35 × 25–35	Slight	1–2	1–3	Up to 44 × 3.5–6	11–17	5–14	2–>5	15–24 × 13–20	0.5–2	E	NS
<i>koelreuteriae</i>	3	30–40 × 28–39	Slight	1–2.5	1–3	Up to 50 × 5–7	18–30	2–10	2–4	18–35 × 14–25	1–2	E	NS
<i>panamensis</i>	3	21–26 × 20–24	Slight	1–2	2	–	8–29	4–20	1–4	19–28 × 16–22	0.5–1	E	2
<i>thirumalacharii</i>	3	22–36 × 19–30	Slight to moderate	–	>1	Up to 15– 30	–	–	–	–	–	–	–
<i>thwaitesii</i>	3	28–48 × 25–44	Strong	1–3	1–3	Up to 85 × 4.5–9	8–17	3–12(– 16)	2–4	–	–	–	–
<i>toonae</i>	1–4	7.5–40 × 9–30	Strong	1.5–5	1–2	Up to 85 × 4–18	40–60	1.5–6	2–8	20–24 × 17–27	1.2–3.8	E	NS
<i>trevesiae</i>	3	24–37 × 25–36	Slight	0.5–2.5	2–3	Deciduous and short	10–23	2–9	1–2	–	–	–	–

[†]NC = Number of cells in each spore, GP = The number of germ pores (usual) in each spore, NP = Number of projections on each spore, TF = Tip furcation, WT = Wall thickness, SO = Surface ornamentation, E = Echinulate, NS = Not seen.

The results of analysis of dataset 2 (Fig. 10), closely supports the dataset 1 (Fig. 9). From both datasets, it is clear that all the *Nyssopsora* spp. separated together as an independent, formerly not known lineage in *Pucciniales*. The differences in morphology are significant enough for retaining *Nyssopsora* distinct from the members of *Pucciniaceae*. Therefore, a new family *Nyssopsoraceae* is established for all the *Nyssopsora* species having 1–4-celled pedicellate teliospores with conspicuous projections (spines) on their surface. The genera *Nyssopsora* and *Triphragmium*, which were originally assigned to *Sphaerophragmiaceae*, are now excluded (Figs. 9, 10) and support the current concepts of Aime & McTaggart (2020).

Taxonomy

Nyssopsoraceae Sanjay & Raghv. Singh, fam.nov.

Index Fungorum number: IF559434; Facesoffungi number: FoF12772

Etymology – named after the genus *Nyssopsora*.

Spermogonia unknown. Aecia, when present, uredinioid, without paraphyses; aeciospores echinulate. Uredinia subepidermal in origin, erumpent; urediniospores borne singly on pedicels, echinulate, germ pores not seen. Telia subepidermal in origin, erumpent; teliospores borne singly on pedicel, composed of 1–4-cells (mostly 3-celled), spherical to subspherical (1-celled), dumbbell (2-celled), linearly arranged to triquetrous (3-celled), T-shaped to tetrahedron (4-celled), walls pigmented, bearing conspicuous projections, entire or branched at the tips, 1–4 germ pores in each cell, basidia external.

Type genus – *Nyssopsora* Arthur (1906).

Type species – *Nyssopsora echinata* (Lév.) Arthur (1906).

Nyssosoratoonae Sanjay & Raghv. Singh, sp. nov.

Figs 1–8

Index Fungorum number: IF559435; Facesoffungi number: FoF12771

Etymology – species epithet is derived from the name of the host genus.

Diagnosis – Differs from all the *Nyssopsora* spp. due to occurrence of wide range of cells (1–4) and diverse shape in teliospores: spherical to subspherical (1-celled), dumbbell (2-celled), linearly arranged to triquetrous (3-celled), and T-shaped to tetrahedron (4-celled).

Holotype – AMH 10124

Spermogonia and aecidia unknown. Infection spots hypophyllous, blackish to dark blackish brown, velvety, initially marginal, later scattered on lamina, more or less circular to subcircular, later on coalesce to become irregular, necrotic, 1–4 mm diam. Mycelium internal. Colonies hypogenous, subepidermal in origin and erumpent, pulverulent, dark blackish brown when spores are abundant. Uredinia hypophyllous, 1–4 mm diam., formed in early stage of infection and telia at later stage, loosely or densely aggregated, subepidermal in origin, erumpent and exposed early, pulverulent, blackish brown to black. Urediniospore mostly circular to orbicular, oval, 20–24 × 17–24(–27) μm, wall uniformly thick, (1.2–)2–3.5(–3.8) μm, pale yellow to dark brown, surface minutely echinulate, germ pores not seen, pediculate, pedicels up to 16 × 6–9 μm. Telia hypophyllous, 1–4 mm diam., subepidermal in origin and early exposed, densely aggregated in irregular groups, pulverulent, blackish brown to black. Teliospores borne singly on pedicels, 1–4-celled, mostly 3-celled with a single proximal cell and surmounted by two distal cells, 7.5–40 × 9–30 μm, diverse shapes [1-celled (spherical to subspherical): 7.5–27 × 9–25 μm; 2-celled (dumbbell): (20–)30–32(–35) × (14–)18–23(–25) μm, 3-celled (linearly arranged): (30–)32–35(–40) × (15–)20–22(–24) μm; 3-celled (triquetrous): (30–)32–33(–35) × (18–)20–28(–30) μm; 4-celled (T-shaped): (36–)37–38(–40) × (19–)20–26(–28) μm and 4-celled (tetrahedron): (28–)30–33(–35) × (18–)19–24(–26) μm], wall (1.5–)2.5–4(–5) μm thick, strongly constricted at the septa, initially light brown, dark brown to blackish brown at maturity; with approx 40–60 projections on each spore, mostly 2–8-furcated at tip, 1.5–6 μm long; often barely visible 1–2 germ pores in each cell, appearing near or at the inner angles; pedicels subhyaline to olivaceous, smooth to striated, persistent, up to 85 μm long, (4–)6–10(–18) μm wide.

Material examined – India, Uttarakhand, Chamoli, Govindghat, 30.6185°N, 79.5617 E, on living leaves of *Toona sinensis* (Juss.) M. Roem. (*Meliaceae*), December 2018, coll. Sanjay Yadav, AMH 10124 (holotype), MH-BHU 6 (isotype).

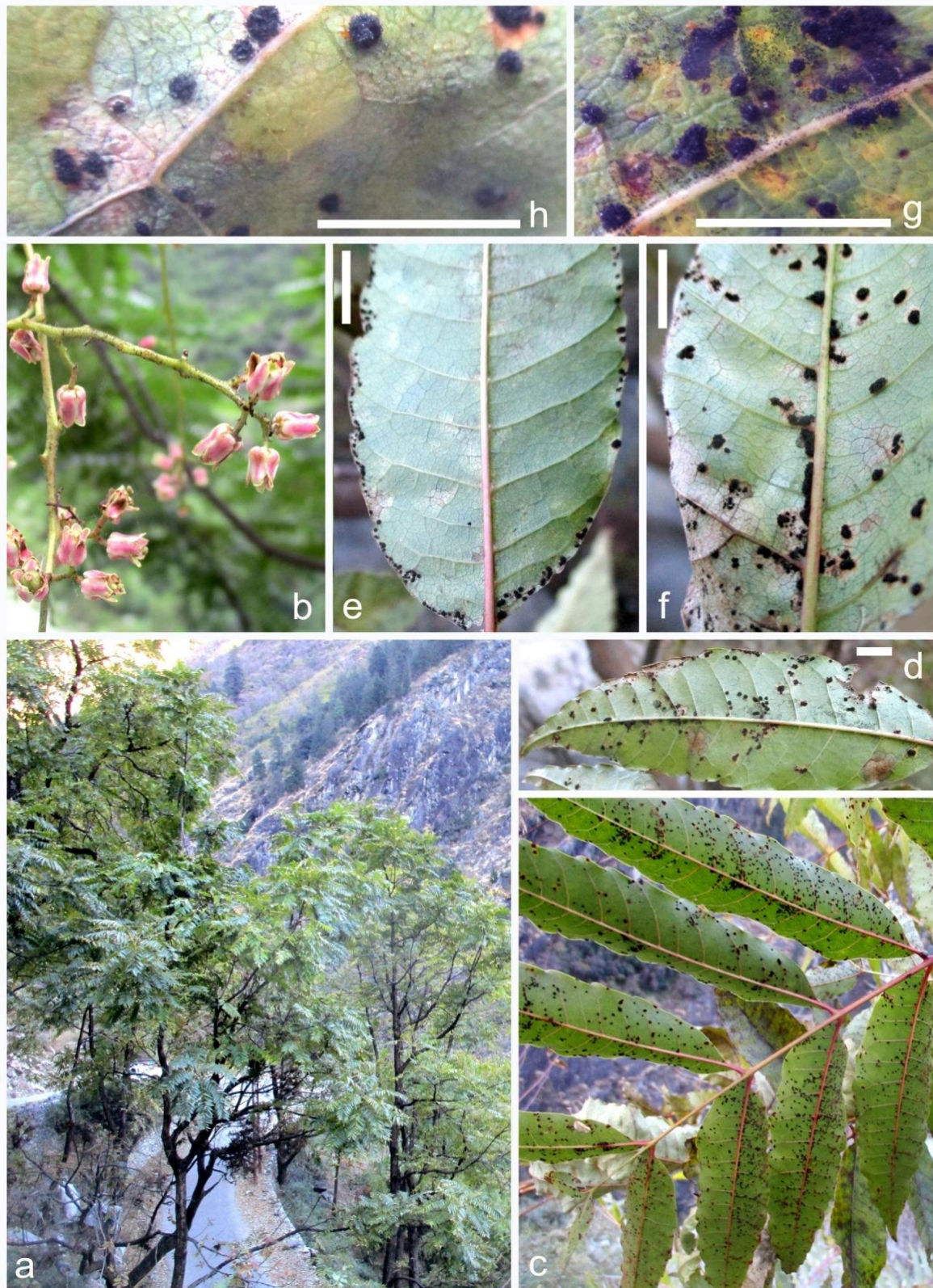


Fig. 1 – Symptoms of *Nyssopsora toonae* on *Toona sinensis* (AMH 10124, holotype). a, b Host Plant in natural habitat. c, d Rust pustules on the lower surface of leaf. e Symptom limited to leaf margin. f Symptom mostly limited to midrib or vein. g, h Close-up of leaf surface showing telia. Scale bars = 10 mm.

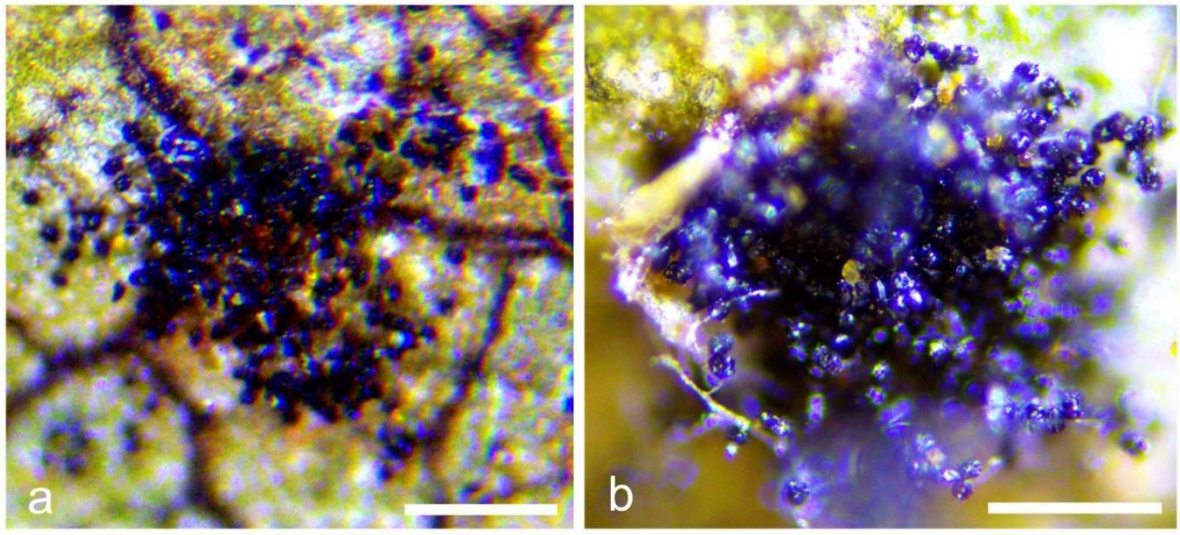


Fig. 2 – *Nyssopsora toonae*, microphotographs (AMH 10124, holotype). a–b Telia on leaflets of *Toonasinensis*. Scale bars: a = 10 μ m, b = 20 μ m.

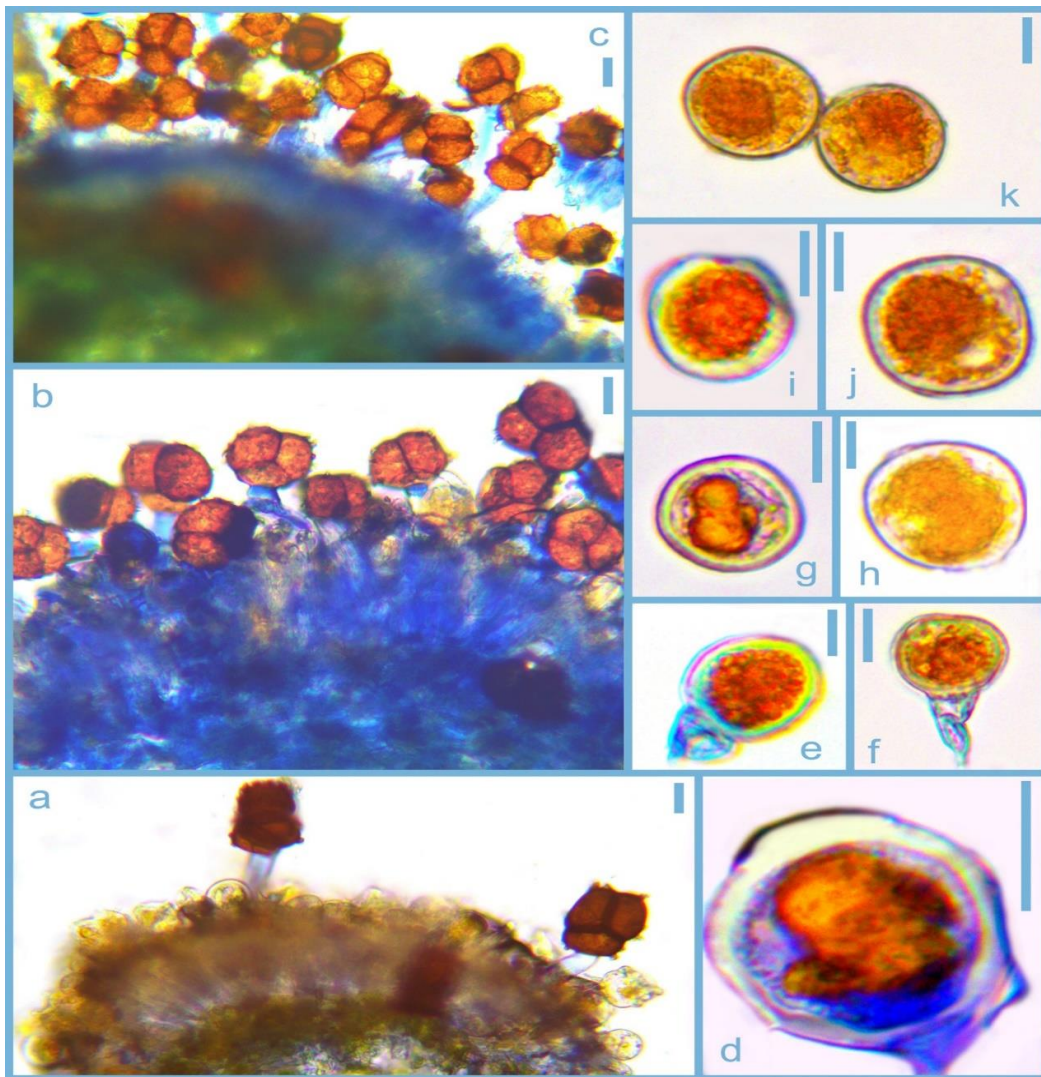


Fig. 3 – *Nyssopsora toonae*, microphotographs (AMH 10124, holotype). a Transverse section of uredinium with two mature teliospores. b, c Transverse section of telium. d–f Stalked Urediniospores. g–k Urediniospores. Scale bars = 10 μ m.

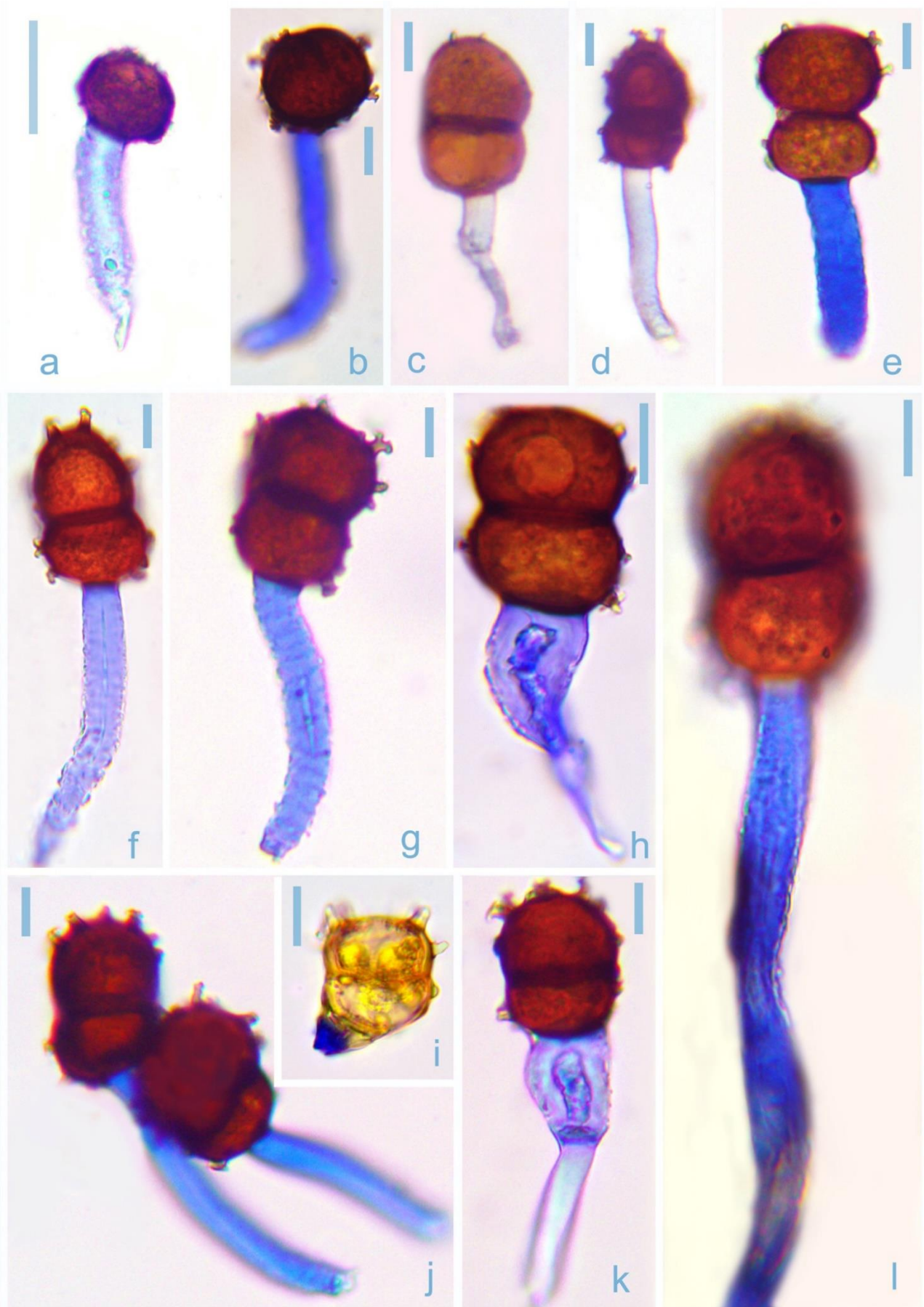


Fig. 4 – *Nyssopsora toonae*, microphotographs (AMH 10124, holotype). a, b 1-celled teliospores. c–l Different forms of 2-celled teliospores. Scale bars = 10 μ m.

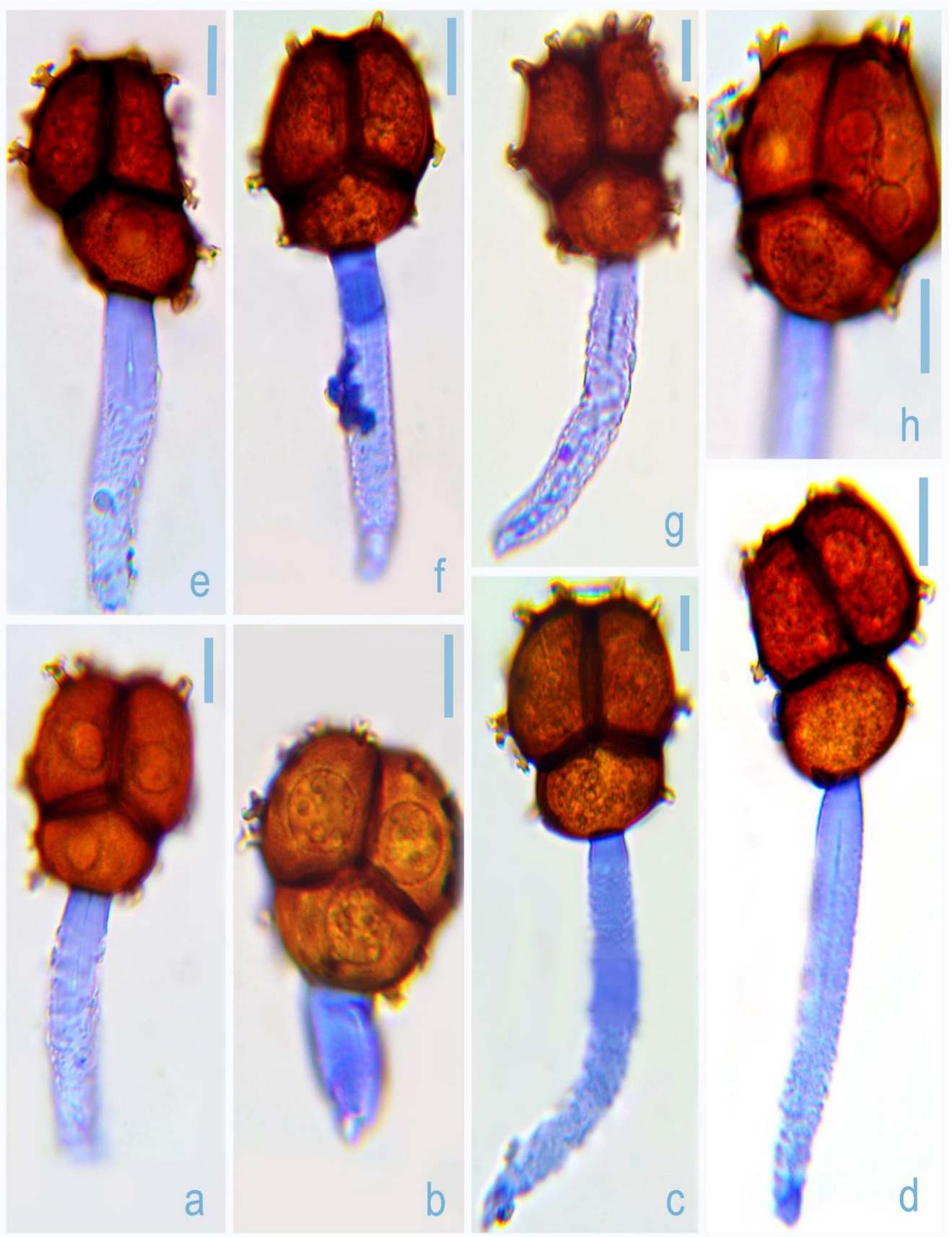


Fig. 5 – *Nyssopsora toonae*, microphotographs (AMH 10124, holotype). a–h Different forms of 3-celled triquetrous teliospores. Scale bars = 10 μ m.

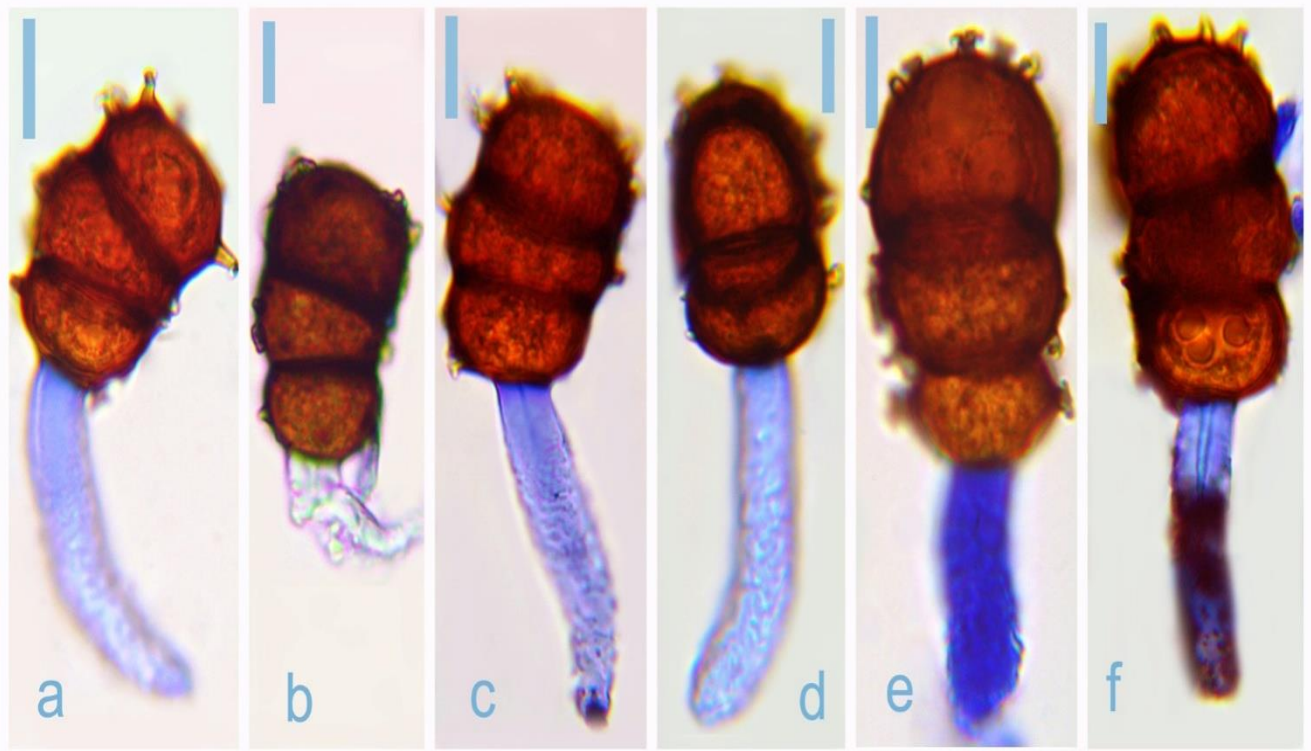


Fig. 6 – *Nyssopsora toonae*, microphotographs (AMH 10124, holotype). a–f Different forms of 3-celled linearly arranged teliospores. Scale bars = 10 μ m.

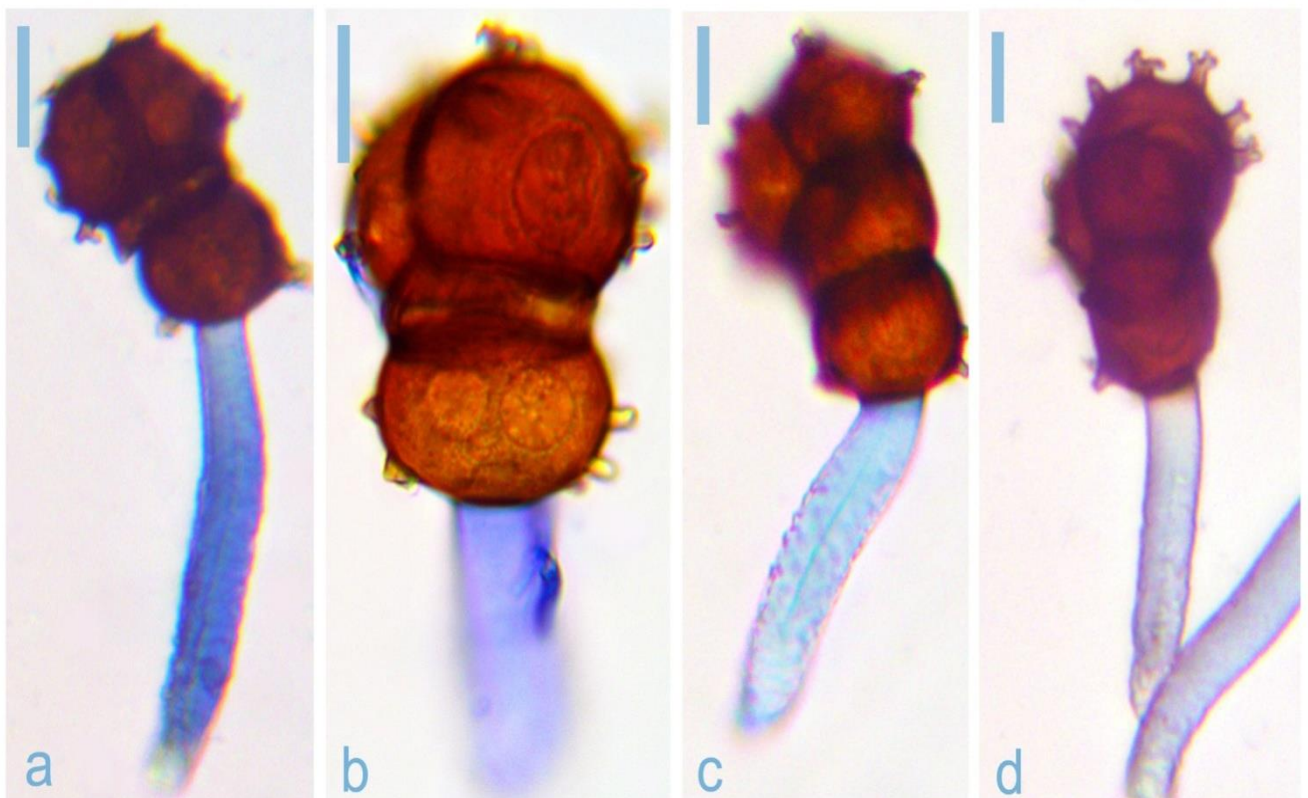


Fig. 7 – *Nyssopsora toonae*, microphotographs (AMH 10124, holotype). a–c Different forms of 4-celled T-shaped teliospores. d 4-celled tetrahedron teliospore. Scale bars = 10 μ m.

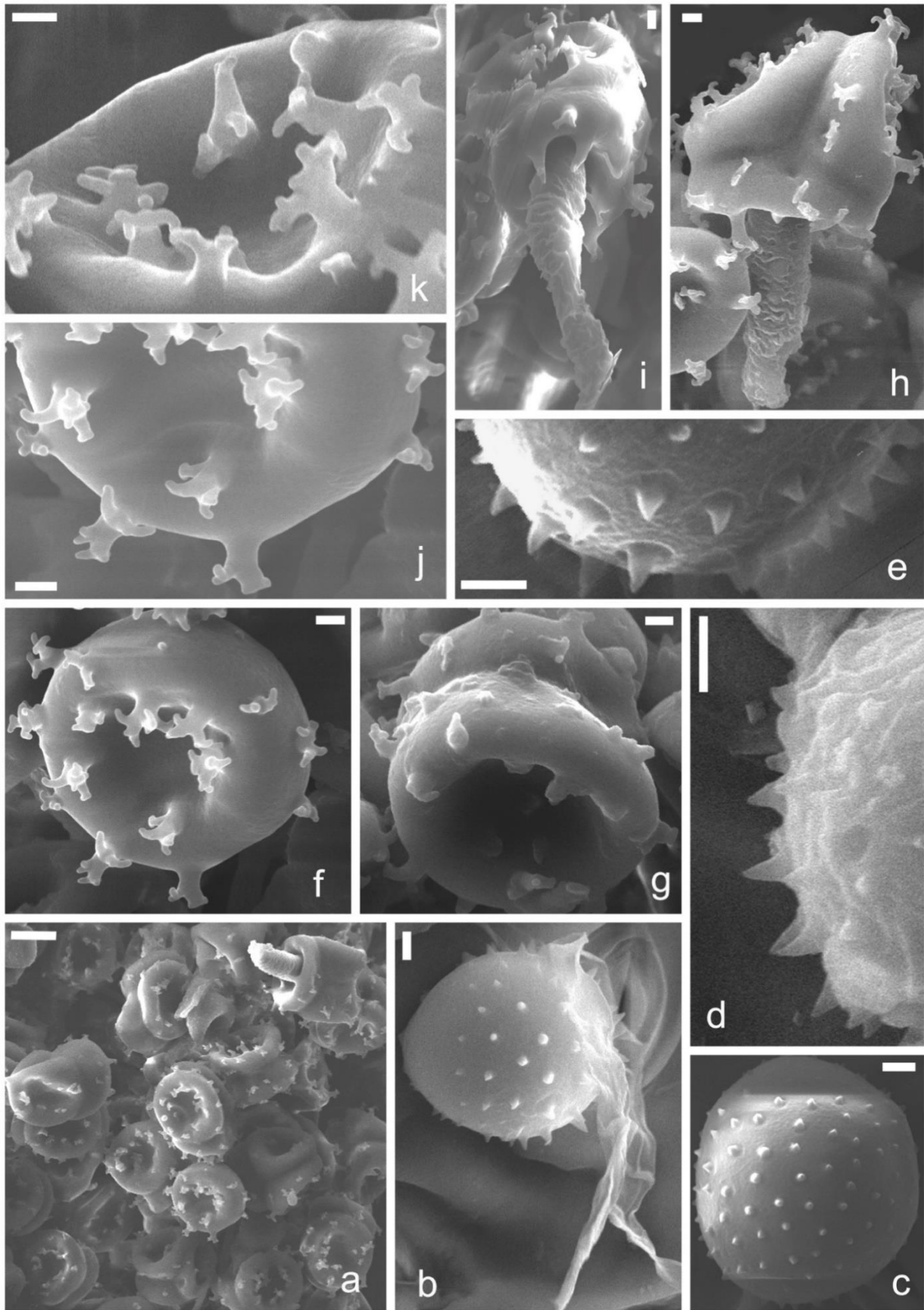


Fig. 8 – SEM characteristics of *Nyssopsora toonae* (AMH 10124, holotype). a Telium. b, c Urediniospore showing echinulate surface. d, e Different patterns of echinulation on the surface of urediniospores. f 1-celled teliospore. g 2-celled teliospore. h 3-celled teliospore. i 4-celled teliospore. j, k Projections on teliospores. Scale bars: a = 10 μ m, b–j = 2 μ m.

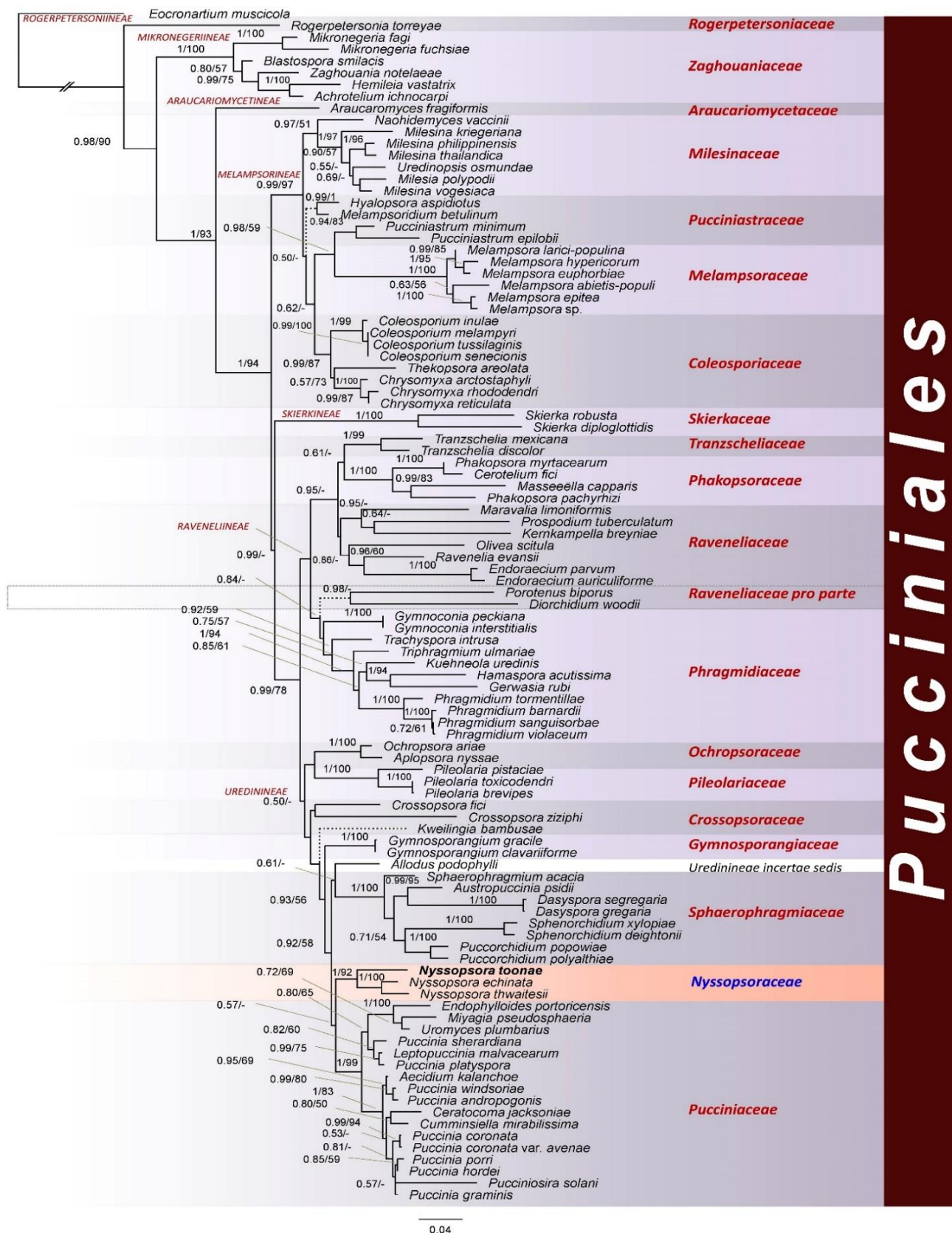


Fig. 9 – Consensus phylogram (50% majority rule) resulting from a maximum likelihood of the combined three-gene (dataset 1: LSU-SSU-CO3) sequence alignments. The Bayesian posterior probabilities (≥ 0.50 ; BI-PP) and maximum likelihood bootstrap support values ($\geq 50\%$; ML-BS) are given at the nodes (BI-PP/ML-BS). All taxa names are written in black, newly introduced species is represented in bold and novel family denoted in blue. The tree is rooted with

Eocronartium muscicola. Families are indicated by coloured blocks; dashed lines indicate uncertainty at the referenced nodes.



Fig. 10 – Consensus phylogram (50% majority rule) resulting from a maximum likelihood of the combined four-gene (dataset 1: LSU-SSU-CO3-ITS) sequence alignments. The Bayesian posterior probabilities (≥ 0.50 ; BI-PP) and maximum likelihood bootstrap support values ($\geq 50\%$; ML-BS)

are given at the nodes (BI-PP/ML-BS). All taxa names are written in black, newly introduced species is in bold and novel family denoted in blue. The tree is rooted with *Eocronartium muscicola*. Families are indicated by coloured blocks; dashed lines indicate uncertainty at the referenced nodes.

Discussion

In light of current concept (Aime & McTaggart 2020), the present phylogenetic analyses used more species and genera, including *Nyssopsora* and *Triphragmium*, to find out the exact placement of all the *Nyssopsora* spp. in *Pucciniales*. From both the datasets it is clear that all the *Nyssopsora* spp. are clustered together and separated as an independent lineage with strong statistical supports, not known in *Pucciniales*. Hence, it is justified to introduce a new family *Nyssopsoraceae* to accommodate all the *Nyssopsora* and allied species having 1–4-celled pedicellate teliospores bearing conspicuous projections (spines) on its surface (Figs 9, 10). Moreover, the members of *Nyssopsoraceae* are separated as a sister lineage of *Pucciniaceae* Chevall. with very low statistical supports (Figs 9, 10). Members of *Pucciniaceae* can be easily distinguished from *Nyssopsoraceae* in having typically with 1 or 2 celled teliospores (Aime & McTaggart 2020).

The type species of the genus *Triphragmium*, *T. ulmariae* (DC.) Link clustered within the *Phragmidiaceae* clade (Figs 9, 10) and was already transferred to this family by Maier et al. (2003). The genera *Nyssopsora* and *Triphragmium*, which were originally assigned to *Sphaerophragmiaceae*, are now excluded (Figs 9, 10) and support the current concepts of Aime & McTaggart (2020).

A total of 12 valid species of *Nyssopsora* have been reported across the world until now based on morphological data alone (Ngachan & Goswami 1985, Lohsomboon et al. 1990, Baiswar et al. 2014, Carvalho et al. 2014, Phetruang et al. 2019, Tykhonenko et al. 2021). Molecular sequence data of all species are necessary in order to get taxonomically sound decisions, but limited molecular sequence data are available viz., *N. echinate* (SSU), *N. koelreuteriae* (ITS) and *N. thwaitesii* (SSU, LSU & ITS) (Swann & Taylor 1995, Wingbeld et al. 2004, Baiswar et al. 2014).

Nyssopsora cedrelae and *N. toonae* both are reported on the same host species *Toona sinensis* (\equiv *Cedrela sinensis*), but the former species differs as later have wide range of cells (1–4) and diverse forms of shape in teliospores: spherical to subspherical (1-celled), dumbbell (2-celled), linearly arranged to triquetrous (3-celled), and T-shaped to tetrahedron (4-celled). Such features are not reported to any species of the *Nyssopsora* (Tables 2, 3).

Analytical studies recognize, *N. toonae* as a separate and undescribed species showing strong support of its taxonomic position within genus *Nyssopsora*. With blast search in GenBank, no sequence identical to any of the investigated genes of this species was encountered. However, the formation of wide range of cells (1–4) and the diverse forms of shape in teliospores (spherical to subspherical, dumbbell, linear to triquetrous and T-shaped to tetrahedron shaped) are the additional striking features of *N. toonae* that easily distinguish it from other *Nyssopsora* spp. (Table 2).

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Table 3 Spore states, hosts and distribution of *Nyssopsora* species.

<i>Nyssopsora</i> spp.	State	Host families	Hosts	Distribution	References
<i>asiatica</i>	III	<i>Araliaceae</i>	<i>Acanthopanax sciadophylloides</i> , <i>Aralia chinensis</i> var. <i>canescens</i> , <i>A. chinensis</i> var. <i>glabrescens</i> , <i>A. cordata</i> , <i>A. elata</i> , <i>A. spinosa</i> , <i>Evodiopanax innovans</i> , <i>Kalopanax innovans</i> , <i>Merrillioanax listeri</i>	East Asia, U.S.S.R.	Ito (1950), Tai (1979), Lohsomboon et al. (1990)
<i>cedrelae</i>	I, II, III	<i>Anacardiaceae</i> <i>Meliaceae</i> <i>Simaroubaceae</i>	<i>Ailanthus altissima</i> , <i>Cedrela serrata</i> , <i>C. sinensis</i> , <i>Cedrela</i> sp., <i>Choerospondias axillaris</i>	South Asia, East Asia	Ito (1950), Lütjeharms (1937), Tai (1979), Sydow & Sydow (1912)
<i>citriobati</i>	III	<i>Pittosporaceae</i>	<i>Citriobatus pauciflorus</i> , <i>C. multiflorus</i>	Eastern Australia	Sydow (1938)
<i>clavellosa</i>	III	<i>Araliaceae</i> <i>Rosaceae</i>	<i>Aralia nudicaulis</i> , <i>A. racemosa</i> , <i>Prunus</i> sp.	North America	Arthur (1934), Lütjeharms (1937), Zeller (1935)
<i>echinata</i>	III	<i>Apiaceae</i>	<i>Coelopleurum gmelini</i> , <i>Conioselinum scopulorum</i> , <i>C. pacificum</i> , <i>C. scopulorum</i> , <i>Ligusticum apiodorum</i> , <i>L. filicinum</i> , <i>L. leibergii</i> , <i>L. mutellina</i> , <i>L. porteri</i> , <i>L. prpureum</i> , <i>L. scopulorum</i> , <i>Ligusticum</i> sp., <i>Meum athamanticum</i> , <i>M. mutellina</i> , <i>Oenanthe californica</i> , <i>O. sarmentosa</i> var. <i>californica</i> , <i>Selinum pacificum</i>	Europe, North America	Lütjeharms (1937), Sydow & Sydow (1912), Wilson & Henderson (1966)
<i>eocaenica</i>	III	–	Sakhalinian amber of Eocene	East Asia	Tykhonenko et al. (2021)
<i>formosana</i>	II, III	<i>Sapindaceae</i>	<i>Koelreuteria bipinnata</i> , <i>K. formosana</i>	East Asia	Lütjeharms (1937), Sawada (1931)
<i>koelreuteriae</i>	II, III	<i>Sapindaceae</i>	<i>Koelreuteria bipinnata</i> , <i>K. paniculata</i> , <i>Koelreuteria</i> sp.	East Asia	Sydow & Sydow (1912), Lohsomboon et al. (1990)
<i>panamensis</i>	II, III	<i>Anacardiaceae</i>	<i>Astronium graveolens</i>	Central America	Carvalho et al. (2014)
<i>thirumalacharii</i>	III	<i>Araliaceae</i>	<i>Brassaiopsis griffithii</i>	South Asia	Ngachan & Goswami (1985)
<i>thwaitesii</i>	III	<i>Araliaceae</i> <i>Rubiaceae</i>	<i>Brassaiopsis hainla</i> , <i>Hedera vahlii</i> , <i>Heptapleurum ellipticum</i> , <i>H. stellatum</i> , <i>H. venulosum</i> , <i>Heptapleurum</i> sp., <i>Schefflera bengalensis</i> , <i>S. elliptica</i> , <i>S. leucantha</i> , <i>S. lucescens</i> , <i>S. odorata</i> , <i>S. polybotrya</i> , <i>S. roxburgii</i> , <i>S. scandens</i> , <i>S. stellata</i> , <i>S. venulosum</i> , <i>S. wallichiana</i> , <i>Schefflera</i> sp., <i>Neonauclea bartlingii</i>	South Asia, South-east Asia	Monson (1974), Sydow (1921), Tai (1979), Berkeley & Broome (1875), Ngachan & Goswami (1985), Bagyanarayana et al. (1987), Baiswar et al. (2014), Lohsomboon et al. (1990), Phetruang et al. (2019)
<i>toonae</i>	II, III	<i>Meliaceae</i>	<i>Toona sinensis</i>	South Asia	In this communication
<i>trevesiae</i>	III	<i>Araliaceae</i>	<i>Trevesia sundaica</i>	South-east Asia	Boedijn (1959), Gäumann (1921)

I = Aecial state, II = Uredinial state, III = Telial state

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