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Microthia nepenthis, a new combination for *Zythia nepenthis*

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A new combination is provided for *Zythia nepenthis*, a fungus repeatedly recorded on various *Nepenthes* plants in a greenhouse in Prague and successfully isolated from stem rot and conidial tendrils. Phenotypic data including host specificity matches the description of *Z. nepenthis*. Based on sequences of ITS rDNA and the gene for β-tubulin, this species was placed in *Microthia*, which corresponds with the generic concept of *Microthia*.

Key words: asexual morph, ITS, 28S and 18S rDNA, β-tubulin, Sordariomycetes, *Cryphonectriaceae*.

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Nová kombinace je navržena pro druh *Zythia nepenthis*, opakovaně zaznamenaný na různých rostlinách rodu *Nepenthes* ve skleníku v Praze a úspěšně izolovaný jak z hniloby stonku, tak z konidiálních kupek. Fenotypická data spolu s vazbou na *Nepenthes* jsou v souladu s popisem druhu *Z. nepenthis*. Na základě sekvencí ITS rDNA a genu pro β-tubulin byl tento druh zařazen do rodu *Microthia*, což rovněž odpovídá i jeho rodovému konceptu.

SHORT TAXONOMIC REPORT

M a t e r i a l a n d m e t h o d s. Strains were isolated from conidial tendrils produced from conidiomata immersed in necrotised leaf tissue of *Nepenthes truncata* Macfarlane and *N. truncata* × *veitchii* Hook. f. and from necrotised stems of diseased plants of a *Nepenthes* sp. grown in the greenhouse of the Department of Experimental Plant Biology in the Botanical Garden of Charles University, Faculty of Science (Prague, Czech Republic). Slides for microscopy were

mounted in tap water, 3% KOH and Melzer reagent. Microscopic structures were examined and documented with differential interference contrast (Olympus BX-51 with digital camera, Quick Photo software, Olympus, Japan). Representative specimens were deposited in PRC (Herbarium of Charles University, Prague) and living strains in CCF (Culture Collection of Fungi, Charles University, Faculty of Science, Prague; Tab. 1).

Tab. 1. Living cultures included in this study, DNA regions sequenced and their GenBank accession numbers.

Living culture	Herbarium voucher	Host plant	18S rDNA	ITS1-5.8S-ITS2 rDNA	28S rDNA	β-TUB
CCF 5734	–	*Nepenthes* sp.	LS973976	LS973979	LS973983	LS973987
CCF 5735	–	*Nepenthes* sp.	LS973977	LS973980	LS973984	LS973988
CCF 5723	PRC 3988	*N. truncata*	LS973978	LS973981	LS973985	LS973989
CCF 5935	PRC 4116	*N. truncata* × *veitchii*	–	LS973982	LS973986	LS973990

DNA was extracted from 2-week old colonies using a Zymo Research Fungal/Bacterial Kit (Zymo Research, Orange, USA). Nuclear rDNA containing the ITS1-5.8S-ITS2 regions including partial 28S was amplified with primer set ITS1F/NL4 (Gardes & Bruns 1993, White et al. 1990), the 18S region with primer set NS1/NS4 (White et al. 1990) and a fragment of the gene encoding β-tubulin (β-TUB) with primer set T1/T22 (Glass & Donaldson 1995, O'Donnell & Cigelnik 1997). The PCR products were viewed by means of electrophoresis on 1% (w/v) TAE agarose gel and stained with ethidium bromide. The PCR products were purified with the Gel/PCR DNA Fragments Extraction Kit (Geneaid Biotech, Bade City, Taiwan). Both strands of the PCR fragments were sequenced with the primers used for amplification in the Sequencing Laboratory of the OMICS Core Facility, BIOCEV (Vestec, Czech Republic) or Macrogen Sequencing Service (Amsterdam, the Netherlands). Forward and reverse reads were assembled in Geneious 6.1.5 software (Biomatters, Auckland, New Zealand) and consensual sequences were produced. All sequences were deposited in GenBank (Tab. 1).

BLAST search (Altschul et al. 1990) in the GenBank database performed with sequences from all regions indicated affinity of the fungus under study to the genus *Microthia* Gryzenh. & M.J. Wingf., particularly to *M. havanensis* (Bruner) Gryzenh. & M.J. Wingf. Due to the very low variability of 28S (differing only 2 bp from *M. havanensis*) and 18S rDNA (identical to several *Cryphonectria* species), only two datasets were assembled from sequences of ITS rDNA and β-TUB originating from the studies of Gryzenhout et al. (2006) and Bragança et al. (2011). Alignments were performed using the MAFFT algorithm implemented in the

Geneious 6.1.5 software and manually edited in the same software to increase the homology. Because the two datasets were incongruent (distinctly different topologies were obtained from single-gene analyses by visual inspection), they were analysed separately. Phylogenetic analyses were performed with Bayesian inference using MrBayes version 3.2 (Ronquist et al. 2012) and with Maximum likelihood analysis using the RAxML Web Server version 7.7.1 (Stamatakis et al. 2008) accessed through the CIPRES Science Gateway (Miller et al. 2010). Bootstrap support values for the maximum likelihood tree were obtained using the RAxML rapid bootstrap algorithm with 100 replicates. For the Bayesian analysis, the best-fit models were determined using jModeltest version 2.1.5 (Darriba et al. 2012). TIM2ef+I+G and TPM1uf+G were the models for the ITS and β-TUB regions, respectively. Two independent runs of 4,000,000 generations were run and sampled every 100th generation, with the first 25% of tees discarded as burn-in. Posterior probabilities (PP) were used as Bayesian branch support for consensus trees. The average standard deviation of split frequencies estimating convergence reached the level of 0.003 at the end of both the analyses.

Microthia nepenthis (Henn.) Koukol & Hrabětová, **comb. nov.** Fig. 3
(MycoBank MB 826886)

Basionym: *Zythia nepenthis* Henn., Hedwigia 44: 173 (1905)

Description on natural substrate. Conidiomata immersed, stromatic, unilocular, subsphaerical, emerging through host tissue with ostioles covered by orange crystals. Conidiophores mostly unbranched, without paraphyses, up to 30 µm long, terminated by a phialide, 8.5–15 µm long and 1.5–2.5 µm wide. Conidia produced as pale orange droplets or tendrils, hyaline, cylindrical with rounded ends, aseptate, 2.5–4.5 µm long and 1–2 µm wide. Sexual morph not observed.

Description in culture. Colonies on MEA and PDA fluffy to floccose, white to light orange, producing dense white hyaline to light orange aerial mycelium. Mycelial growth 5.5 mm/day on MEA and 5.7 mm/day on PDA at 20 °C. Some cultures produced subsphaerical, slightly immersed conidiomata 0.4–0.6 mm in diam. producing blackish orange droplets of conidia (Fig. 3h). Phialides and conidia were similar in shape to those on the natural substrate, measuring 5.3–10.5 × 0.9–1.6 µm and 2.2–3.3 × 0.9–1.3 µm, respectively. The cultures tended to become sterile during the cultivation.

Notes. The phenotypic characteristics of the fungus correspond well to the description of *Zythia nepenthis* (Hennings 1905) and illustration provided by Kolkowitz et al. (1905). The most distinct feature was the production of orange conidial drops or tendrils from ostioles emerging from decolourised plant tissue.

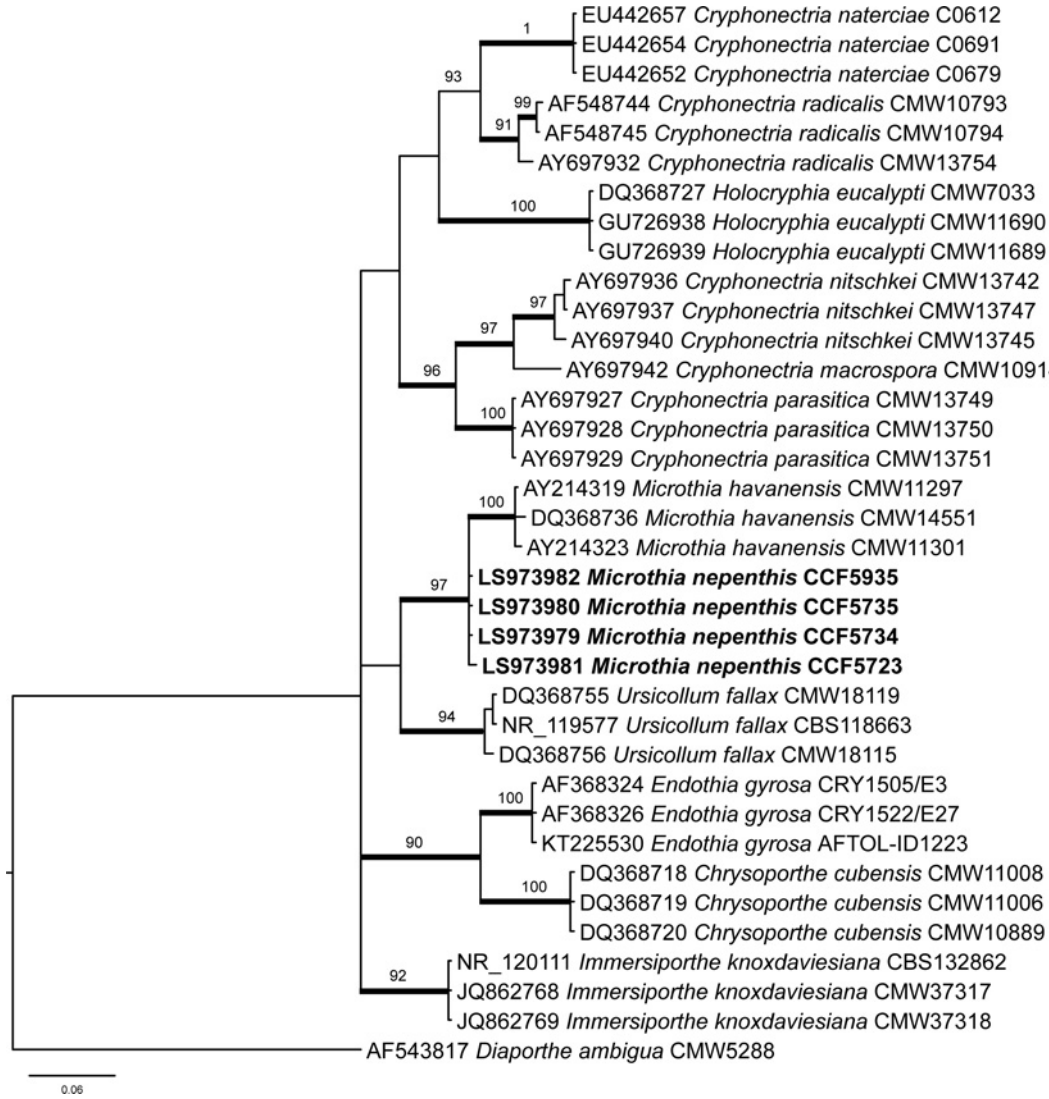

Fig. 1. Phylogenetic hypothesis showing placement of *Microthia nepenthis* based on analysis of ITS rDNA. Numbers above branches represent ML bootstrap support values BS > 90%, thick lines at branches indicate PP > 0.5.

The type specimen could not be located, but morphology and dimensions of conidiomata, conidiogenous cells and conidia and host affinity to *Nepenthes* mentioned in the description of *Z. nepenthis* (Hennings 1905) matched the characteristics of the fungus.

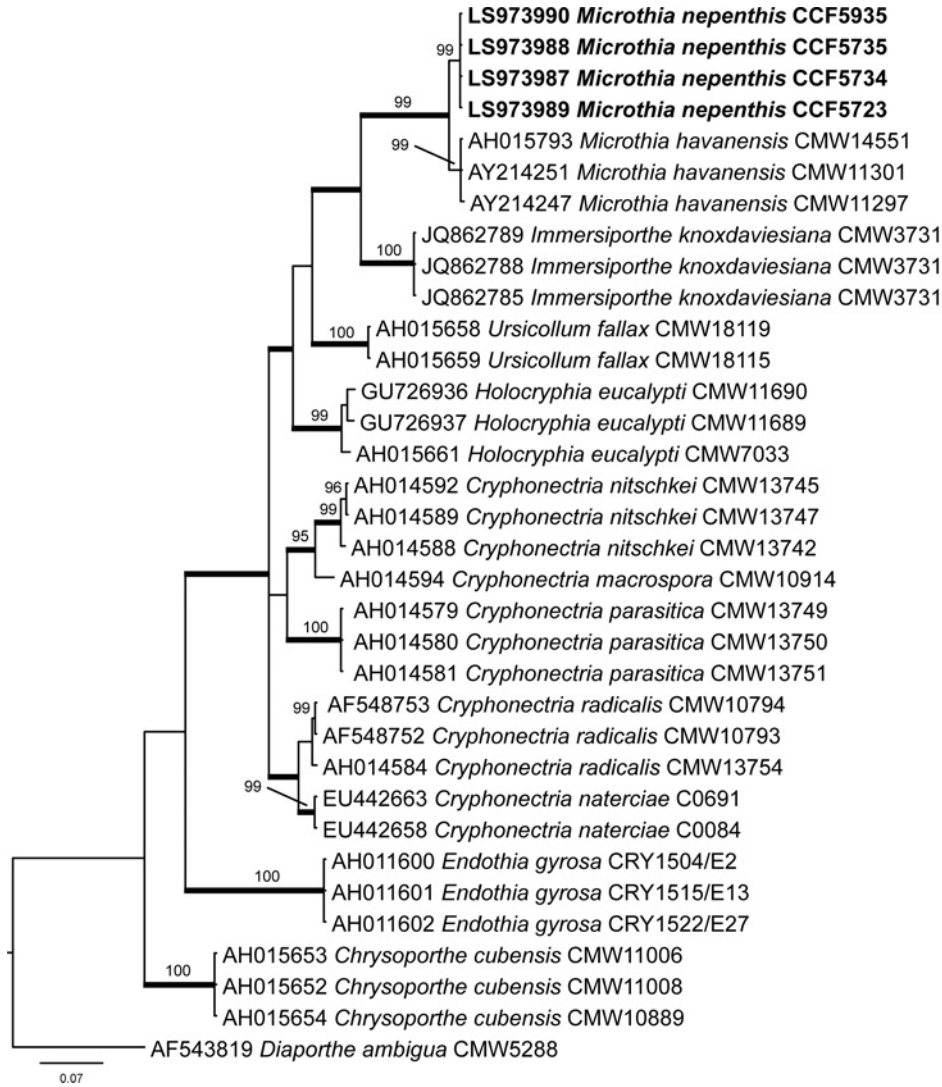

Fig. 2. Phylogenetic hypothesis showing placement of *Microthia nepenthis* based on analysis of gene for β-tubulin. Numbers above branches represent ML bootstrap support values BS > 90%, thick lines at branches indicate PP > 0.5.

Zythia nepenthis was first briefly documented from dead leaves of *Nepenthes bicalcarata* from the Botanical Garden in Berlin in 1904 by Hennings (1905), who considered this species a parasite. Since that time, no records of this fungus have been published from nature or other plant cultures. Recently, numerous *Nepenthes*

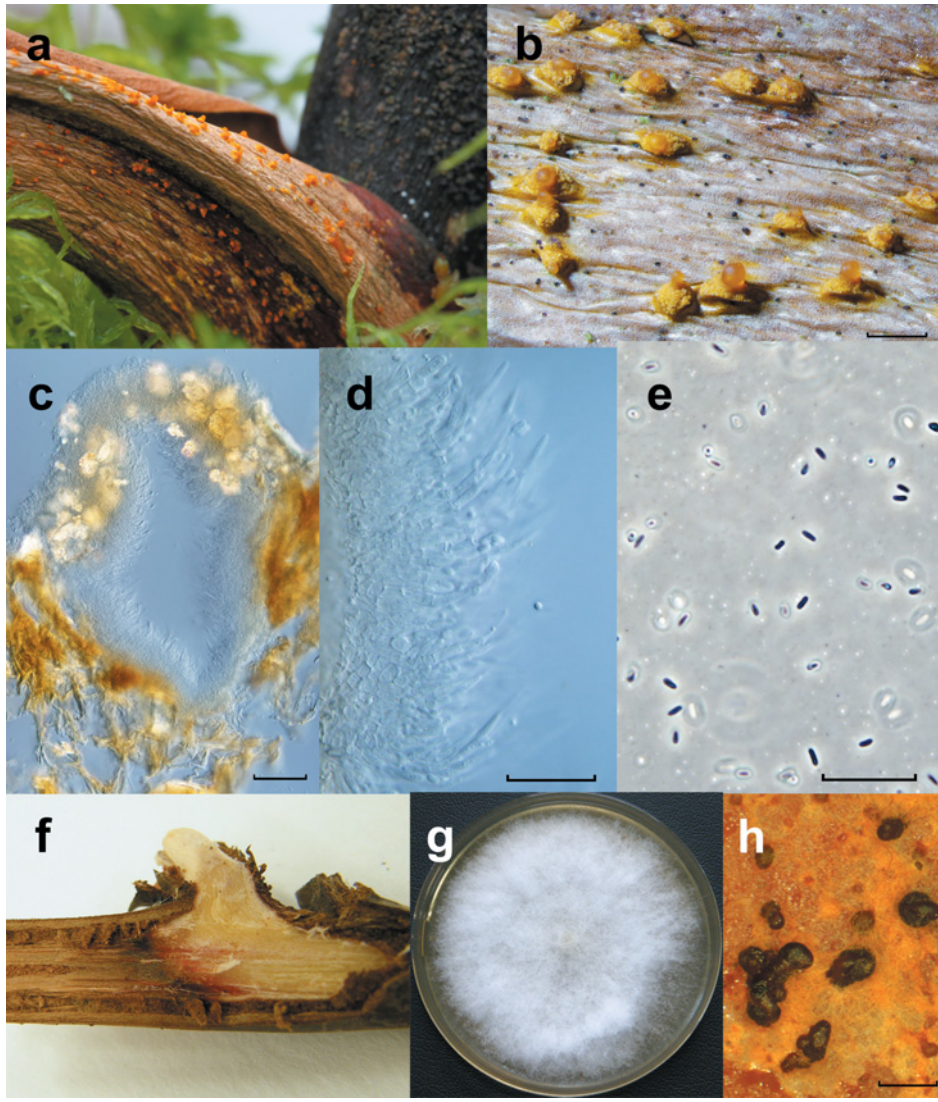

Fig. 3. *Microthia nepenthis* (PRC 3988 a–e, CCF 5734 g–h): **a** – necrotised plant leaf with numerous emerging orange conidiomatal ostioles and conidial drops; **b** – detail of ostioles; **c** – cross section of pycnidium; **d** – conidiogenous cells; **e** – conidia; **f** – stem rot of *Nepenthes* sp.; **g** – mycelium on MEA after 1 wk; **h** – conidiomata with droplets of conidia produced by fresh cultures on MEA. Scale bars = 500 µm (b), 50 µm (c), 20 µm (d, e), 100 µm (h). Photo by O. Koukol (a–e) and K. Černý (f–h).

plants in the greenhouse of the Department of Experimental Plant Biology, Charles University, Faculty of Science showed similar symptoms of stem and leaf blight, basal stem rot and finally wilting and dying of particular shoots or entire plants. The same fungal species was isolated from diseased or dead petioles and stems of different *Nepenthes* species in three consecutive years.

Molecular data placed the isolated strains into the well-supported lineage of *Cryphonectriaceae* (Sordariomycetes) as a sister species to *Microthia havanensis* based on both ITS rDNA and β-TUB analyses (Figs. 1 and 2). *Microthia* is characterised by orange, submersed conidiomata with cylindrical conidiophores with or without lateral branches, often with long sterile cells, phialidic conidiogenous cells and hyaline, cylindrical, aseptate conidia (Gryzenhout et al. 2006), which agrees well with the morphology of our fungus.

The genus *Zythia* comprises a conglomerate of largely unrelated species and the type of the genus, *Zythia resinae* (Ehrenb.) P. Karst., is currently accommodated in Lecanoromycetes as *Sarea resinae* (Fr.) Kuntze (Hawksworth & Sherwood 1981). Since *Z. nepenthis* matches well the generic concept of *Microthia*, the epithet is combined into the taxonomically correct genus and a new combination for the fungus isolated from *Nepenthes* is provided herein.

The pathogenicity of *M. nepenthis* is currently unknown, which partly reflects the ecologies of the two most closely related species. *Microthia coccolobae* (Vizioli) Gryzenh. & M.J. Wingf. isolated from cankers of *Coccoloba uvifera* (L.) L. was able to induce cankers in the same plant when artificially inoculated, whilst *M. havanensis* occurs mostly on bark of *Eucalyptus* trees and, although it was associated with cankers, is considered saprotrophic by Gryzenhout et al. (2006). Experimental inoculation tests are thus necessary to elucidate the pathogenicity of *M. nepenthis*.

Cultures examined

Czech Republic. Prague, Botanical Garden, greenhouse of the Department of Experimental Plant Biology, Charles University, Faculty of Science, 50°04'16.98" N, 14°25'22.90" E, alt. 223 m, dead leaf petioles of *Nepenthes* sp., 23 Feb 2016, K. Černý 61/16 (living culture CCF 5734), K. Černý 64/16 (living culture CCF 5735); ibid., dead leaf petioles of *Nepenthes truncata*, 3 Mar 2017, O. Koukol Nep1 (PRC 3988, living culture CCF 5723); ibid., dead leaf petioles of *Nepenthes truncata* × *veitchii*, 21 Feb 2018, O. Koukol Mnep2 (PRC 4116, living culture CCF 5935).

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